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Minireview

Ion channels: molecular basis of ion selectivity

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Recent mutagenesis studies of the ion channel proteins have allowed us to identify amino acid residues critical in determining ion selectivity. Ion selectivity of a channel can be altered even by single amino acid substitutions. Functional analyses of mutants largely support views in classical biophysics that the pore size and the fixed charges are major determinants of ion selectivity. For full understanding of the molecular mechanism of ion selectivity, elucidation of the tertiary structure of channel proteins remains essential.

Ion channel; Ion selectivity; Site-directed mutagenesis; Functional expression

1. INTRODUCTION

Ion channels are transmembrane macromolecules that provide an aqueous pore through the lipid bilayer. They are fundamental elements in cellular electrical activity and other functions. In many cases, their biological significance is dependent of the fact that ion channels are not simple pores but exhibit ion selectivity. For example, action potentials would be impossible without strict Na^+ selectivity of the sodium channel. This interesting property has led us to many investigations of various types of ion channels. Combined with classical biophysics, electrophysiological analyses successfully constructed the framework of how the ion channels operate [1].

For ion selection to occur, there must be at least one narrow region within the channel pore where permeating ions come into close contact with the channel protein. Intuitively, we would expect that the size of permeating ions must be a critical factor. The size of ions is, however, a somewhat fuzzy concept, because they are hydrated in solution and probably become partially dehydrated when they pass through the channel. Therefore, structural elements that affect the degree of dehydration should be another determinant of ion selectivity.

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Abbreviations: AChR, acetylcholine receptor; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; GluR, glutamate receptor; NMDA, *N*-methyl-D-aspartic acid.

We can assume that fixed charges in the pore is one of the determinants, since electrostatic interactions between permeating ions and the fixed charges in the pore would provide energy required for dehydration. This assumption leads to an idea that there must be stronger electrostatic forces in a channel that is selective for more heavily hydrated ions, which correspond to ions with smaller crystal radii (for example, Li^+ and Na^+) [2]. Electrostatic interaction is also important for selecting divalent ions over monovalent ions. Ca^{2+} selectivity of the calcium channel has been successfully explained by assuming two negatively charged sites, which attract Ca^{2+} more strongly than monovalent cations [3,4]. As well as at the narrow channel portion, biophysical calculations have suggested importance of fixed charges at the vestibule or mouth of the channel or even at the membrane surface, which would exert an influence on the rate of ion transport by affecting local ionic conditions [5].

Despite the development of these biophysical ideas, we did not know very much about the structural basis of ion selectivity. Furthermore we did not have experimental methods to test the biophysical interpretations of channel functions prior to the introduction of molecular biology to this field. This has revolutionized our approach to the understanding of channel functions [6]. In particular the combination of recombinant DNA and patch-clamp techniques has allowed detailed functional analyses of site-specifically mutated channels. This article deals with the molecular basis of ion selectivity of the ligand-gated channels, which include the nicotinic acetylcholine receptor channel, the glutamate receptor channels, and the voltage-gated sodium, potassium and calcium channels, mainly based on the results of functional studies of mutant channels.

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2. LIGAND-GATED CHANNELS

2.1. *Nicotinic acetylcholine receptor channels*

The nicotinic acetylcholine receptor (AChR) channel is the most extensively studied ligand-gated channel. It is a pentameric transmembrane protein composed of four kinds of homologous subunits, which are assembled in the molar stoichiometry of $\alpha_2\beta\gamma\delta$ and arranged pseudosymmetrically around a central channel. The hydropathy analysis reveals that each subunit has four hydrophobic segments (M1–M4), which presumably form transmembrane α-helices. It is now fairly well established that the M2 hydrophobic segment of each subunit forms at least part of the pore wall, with its amino-terminal end located close to the cytoplasmic side of the membrane. This conclusion is mainly based on functional analyses of site-specifically mutated AChR channels [7,8] and photoaffinity labelling studies [9]. Involvement of other segments, particularly the M1 segment, in forming the pore wall has also been proposed [10].

Recent mutagenesis work has further refined the prediction for the open channel structure [11–13]. The narrow part of the channel is composed of two ring-like clusters of uncharged polar residues ('central ring') and negatively charged and glutamine residues ('intermediate ring') located near the amino-terminal end of the M2 segment. It has not been settled which ring is more critical in wild-type channels (Fig. 1). These results suggest that the narrow part of the channel pore is relatively short, being consistent with the estimate from electrophysiological experiments [14]. Furthermore, arrangement of the subunits at the narrow region are asymmetrical, as judged from the effects of mutations in different subunits. While the δ-subunit mutations are most influential in the central ring, the effects of γ-subunit mutations are the strongest in the intermediate ring. This asymmetry suggests the possibility that the process of ion permeation is composed of multiple steps of interaction with different subunits. The channel pore is larger in cross-section on both sides of the narrow portion. Negatively charged residues which are located at the external and internal mouths ('cytoplasmic and extracellular rings'), probably to attract permeating cations into the pore.

The AChR channel was regarded as a water-filled non-selective pore because the permeability sequence of this channel is like that of free solution mobility of alkali metal cations. However, the single-channel conductance sequence is different from the mobility sequence, indicating that the AChR channel selects between alkali metal cations. Analysis of the effect of mutations, mainly of the residues in the intermediate ring, showed that both the charge and the size of the side chains are important in determining the selectivity [15]. Analysis of permeability of organic cations provided a supplementary idea that the negative charges in the intermediate ring are also important in sustaining the channel pore by electrostatic repulsion between the charged side chains [16].

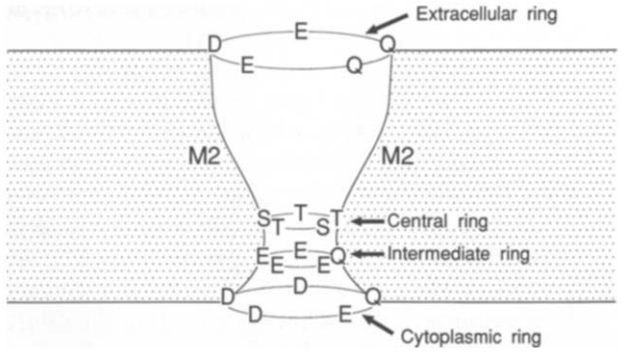

Fig. 1. A proposed open channel structure of the *Torpedo californica* AChR channel, based on mutagenesis work [7,11,15,16]. The position of three anionic rings and one uncharged polar ring are indicated. Amino acid residues in the four rings are represented by the one-letter code. This is probably the highest 'resolution' that can be attained from functional studies of site-specifically mutated channels.

Recently, successful conversion to an anion-selective channel of the neuronal nicotinic acetylcholine receptor, which is a homomeric channel of the α-7 subunit, has been reported [17]. The mutations involved substitution of alanine for the glutamic acid residue in the intermediate ring, and insertion of proline on the amino-terminal side of the substituted alanine. Although it is still premature to propose a detailed molecular basis for anionic selectivity, it appears that slight changes of the geometry of the M2 segment are related to anion selectivity. The lysine residue located on the carboxyl-terminal side of the substituted alanine, which is well conserved in the AChR subunits, may become exposed to cause the anion selectivity.

2.2. *Glutamate receptor channels*

Excitatory synaptic transmission in the mammalian central nervous system is mediated predominantly by glutamate receptor (GluR) channels. Glutamate receptors are classified by their sensitivity to different agonists into AMPA, kainate and NMDA receptors. Recent cloning and expression experiments have characterized numbers of GluR subunits, and their classification according to amino acid homology has in turn correlated well with drug sensitivity [18]. Their predicted primary structures show general structural similarity to the nicotinic AChR subunits, but the GluR subunits possess larger amino- and carboxyl-terminal ends.

Expression of the AMPA/kainate receptor subunits revealed that Ca^{2+} permeability depends on the subunit composition of the receptor. GluR1, GluR3 and GluR4, in combination with each other or as homomeric channels, generate strongly inward-rectifying receptors permeable to Ca^{2+}, while the Ca^{2+} permeability is

greatly reduced when the GluR2 subunit is a constituent of the receptor complex ([19,20]; see [18] for nomenclature). Comparison of the amino acid sequences of the GluR1–4 subunits showed that GluR2 has an arginine residue in the M2 segment where the other GluR subunits have glutamine. Conversion of arginine to glutamine and glutamine to arginine confirmed that the single site of the arginine/glutamine residue is responsible for inward rectification [19] and Ca^{2+} permeability [20]. Another twist in the pursuit of Ca^{2+} permeability came from the genomic DNA sequence encoding the GluR2 subunit, which was found to harbour a glutamine codon, even though an arginine codon was found in mRNAs. Careful exclusion of other possibilities led to the conclusion that the arginine residue in the M2 segment is introduced by GluR2 mRNA editing [21]. Similar editing takes place in the expression of GluR5 and GluR6, although to a lesser extent [21].

The NMDA receptor is highly permeable to Ca^{2+}. This high Ca^{2+} permeability is thought to have a key role in long-term potentiation, excitotoxic cell death and epilepsy. In addition, the NMDA receptor is sensitive to voltage-dependent Mg^{2+} block. An asparagine residue occupies the position homologous to the site in the M2 segment of the AMPA/kainate receptor that controls divalent cation permeability. Replacement of the asparagine residue with glutamine reduced Ca^{2+} permeability and Mg^{2+} block as well as block by an NMDA receptor channel antagonist, MK-801 [22,23]. These results show that Ca^{2+} permeability and Mg^{2+} blockade are critically dependent on the presence of single asparagine residues in the M2 transmembrane segment.

The position of the residues critical in determining Ca^{2+} permeability of the GluR channel is located almost close to the carboxyl-terminal end of the M2 segment (close to the external side), which does not correspond to the position of the narrowest region of the AChR channel, suggesting that these two channel families have different architectures. Interestingly, a possibility has been proposed that bulky and hydrophobic amino acid residues located at the corresponding position of the AChR channel are involved in channel closure [24].

3. VOLTAGE-GATED CHANNELS

Ligand-gated channels mentioned above are cation selective, but they are not strict in selecting among monovalent cations or divalent cations. Stricter ion selectivity is observed with the sodium, potassium and calcium channels. These voltage-gated channels are considered to be similar in their general structure, in which four repeated units of homology (repeats) or four subunits surround the channel pore. Each repeat or subunit has five hydrophobic segments (S1, S2, S3, S5, S6) and one segment with positively charged residues (S4), which serves as a voltage sensor; those six segments form putative transmembrane segments. It was not clear until recently which part of the repeat or subunit actually forms the pore wall. After careful comparison of amino acid sequences of the voltage-gated channels, Guy postulated that two short segments, SS1 and SS2, located between the S5 and S6 segments, partly span the membrane as a hairpin and that the SS2 segment forms part of the channel lining [25]. This hypothesis has been proven by a series of experimental studies. Single amino acid substitutions in the vicinity of the predicted region abolish sensitivity to channel blockers of the potassium channel (to charybdotoxin) [26] and the sodium channel (to tetrodotoxin and saxitoxin) [27]. Furthermore, mutations in this critical region of potassium channels affect sensitivity to tetraethylammonium from either side of the membrane [28] and alter ion selectivity among K^+, Rb^+ and Cs^+ [29].

To pinpoint the amino acid residues forming the selective filter, we took advantage of the amino acid sequence similarity between the sodium and calcium channels, even though they are distinct with respect to ion selectivity. When the amino acid sequences of the SS1–SS2 regions are aligned, glutamic acid residues, that are conserved in all four repeats of the calcium channel, are replaced by lysine in repeat III and by alanine in repeat IV in the sodium channel. Substitution of glutamic acid for the lysine residue and/or for the alanine residue reduced Na^+ selectivity over K^+ and conferred calcium-channel properties of ion selectivity on the sodium channel [30]. This observation suggests that these amino acid residues form part of the selectivity filter of the sodium and calcium channels. Consistent with this view, it has recently been reported that mutations of glutamic acid residues in a brain calcium channel decrease divalent cation selectivity over monovalent cations [31]. It is interesting that the mutation in repeat III reduced Cd^{2+} sensitivity whereas the other in repeat IV showed little effect, indicating inequality of these glutamic acid residues. More extensive and detailed studies will provide us with a molecular interpretation of the postulated Ca^{2+} selectivity mechanism [3,4].

In the SS2 region, the potassium channel has the amino acid sequence GYG, which is evolutionarily very well conserved and also found in the K^+ transport system of higher plants [32]. When YG was removed from this sequence in order to mimic that of the cyclic nucleotide-gated channels, the amino acid sequences of which reveal a distant ancestral connection to the voltage-gated channels, the potassium displayed little selectivity among monovalent cations [33], again demonstrating that very small differences in the primary structure are responsible for extreme functional diversity.

4. CONCLUSIONS

Analyses of ion permeation properties of site-specifically mutated channels have allowed us to speculate about the overall design of the ion channels. (i) The

critical part of the open AChR channel is relatively short. Probably this is also the case for other ion channels. This observation is consistent with a classical biophysical view that the tunnel length must be short to have a high channel conductance [34], although it may difficult to reconcile this with another biophysical idea in which a channel is assumed to accommodate more than one ion (multi-ion models) [1]. The short narrow region means that there is a very large and steep voltage drop across the narrow channel constriction. (ii) Functional analyses of mutated channels show that ion permeation properties, conductance and ion selectivity, are determined by interactions between a limited number of amino acid residues and permeating ions. It is possible to alter ion selectivity by relatively small changes in the primary structure. (iii) Classical biophysics stressed the importance of the pore size and the electrostatic interaction in determining ion selectivity of the ion channels. Functional studies have by and large supported this view.

Our knowledge of the molecular mechanism of ion permeation is still very limited and fragmentary. Experimental results, however, set constraints to model systems. In addition to the extension of these studies, structure–function studies of other types of channel protein, which include an epithelial amiloride-sensitive sodium channel [35], various types of chloride channels [36] and others, will be of particular interest, although elucidation of the tertiary structure of channel proteins remains essential [37], and computational approaches will have to be involved for an understanding of the ion selectivity mechanism.

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