Minireview

Roles of the histidine and tryptophan side chains in the M2 proton channel from influenza A virus

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Abstract The M2 protein form influenza A virus forms a tetrameric ion channel, which enables proton passage across biological membranes when the N-terminal side is acidified. Among the amino acid residues in the transmembrane domain of the M2 protein, His37 and Trp41 are essential for the pH-regulated proton conductance. Current knowledge about the structures and interactions of His37 and Trp41 suggests a model for the M2 ion channel, in which the channel is closed by a network of His37 hydrogen bonds at neutral pH and is opened by a His37-Trp41 cation–π interaction at acidic pH.

Key words: Ion channel; Histidine; Tryptophan; Cation–π interaction; Hydrogen bond; UV Raman spectroscopy

1. Introduction

The M2 protein of influenza A virus forms a proton-specific transmembrane (TM) ion channel, which is activated at acidic pH and plays important roles in both early and late stages of virus infection [1–5]. The polypeptide chain of the M2 protein is composed of 97 amino acid residues with a putative single TM domain in its N-terminal half [6]. The main functional machinery of the M2 ion channel is believed to lie within the TM domain because a synthetic 25-residue peptide (Ser–Ser-Asp-Pro^{25}-Leu-Val-Val-Ala-Ala-Ser^{31}-Ile-Ile-Gly-Ile-Leu-His^{37}-Leu-Ile-Leu-Trp^{41}-Ile-Leu^{43}-Asp-Arg-Leu, M2-TMP) spanning the TM domain (underlined) exhibits proton channel activity similar to that of the full-length M2 protein [7]. Mutagenesis studies have demonstrated that amino acid side chains, in particular those of His37 and Trp41, play crucial roles in the proton conductance. Replacement of His37 by other amino acids impairs the pH sensitivity of channel activity, resulting in a nearly constant membrane current at both neutral and acidic pH [1,2]. On the other hand, mutations of Trp41 to less bulky amino acids such as Ala, Cys, and Phe not only increase the normal inward current from the N-terminal to the C-terminal side of the channel but also permit the reversed outward current, which is suppressed in the wild-type M2 channel [8,9]. These observations suggest that both His37 and Trp41 are essential for formation of the native M2 ion channel that allows proton passage only when the N-terminal ectodomain is exposed to a low pH environment.

An analysis of ion channel activity for mixtures of wild-type M2 protein and its mutants has established that the minimal active unit of the ion channel is a homotetramer [10]. The TM domain alone forms tetramers as revealed by analytical ultracentrifugation [11]. The structure of the TM domain and its arrangement in the tetramer have been studied by using spectroscopic and mutagenesis methods. Circular dichroism spectra have indicated that M2-TMP adopts a predominantly α-helical structure in lipid membrane bilayers [11–13]. Cysteine scanning mutagenesis has suggested that the TM helices are arranged around the channel pore with approximate four-fold rotational symmetry [14]. Solid state nuclear magnetic resonance (NMR) [15–19] and infrared dichroism [20–22] studies have shown that the helix axis in the tetrameric bundle of the TM domain is tilted from the membrane normal (or the channel axis) by 25–38°. In contrast to the substantial knowledge about the main-chain structure of the TM domain and its arrangement in the tetramer, structural information on the amino acid side chains is limited. UV resonance Raman spectroscopy has provided unique information on the structures and interactions of His37 and Trp41 [13].

Raman spectroscopy utilizes inelastic light scattering by molecular vibrations, of which frequencies and modes are sensitive to the structure and interaction around the vibrating atoms. For proteins, Raman signals arise from vibrations of the peptide main chains and amino acid side chains, and even small changes in conformation, ionization, hydrogen bonding, hydrophobic interaction, etc. can be detected as shifts and/or intensity changes of Raman bands [23–25]. In particular, UV resonance Raman spectroscopy is suitable for structural studies of membrane-bound proteins because Raman signals from UV-absorbing amino acid residues such as Trp and His are selectively enhanced, while those from non-absorbing residues and lipid membranes are not enhanced [26,27]. The characteristics of UV resonance Raman spectroscopy were exploited in studying the structures and interactions of His37 and Trp41 in the M2 TM domain incorporated into lipid liposomes [13]. In

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Abbreviations: M2-TMP, a 25-residue peptide encompassing the transmembrane domain of the M2 protein from influenza A virus; TM, transmembrane

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this review, we discuss the M2 ion channel mechanism on the basis of Raman and other structural data on His37 and Trp41.

2. Protonation of His37 and channel activation

Since amino acid His has a pK_a value of ~6.5 and mutations of His37 to other amino acids abolish the transition of proton conductance at pH ~5.8, the activation of the M2 ion channel has long been supposed to be linked with the protonation of His37 [1,2]. However, no direct evidence for the protonation of His37 in the active channel state was reported until recently. The five-membered ring of the His side chain carries two nitrogen atoms and only one of them is protonated in the neutral imidazole form, which is usually stable at pH 7 or higher. At acidic pH, on the other hand, the other nitrogen atom also gains a proton and the ring becomes the cationic imidazolium form. The protonation of His affects the molecular vibrations of the side chain and produces a strong Raman band that can be used as a marker of protonation [27–29]. By using the marker Raman band, the pK_a value of His37 has been investigated for M2-TMP incorporated into liposomes whose lipid composition is identical to that employed for channel activity assays [13]. The Raman titration experiment has clearly shown that the pK_a of His37 is 5.7, which is in good agreement with the pH (5.8) of channel activation [2]. Another important finding by UV Raman spectroscopy is that four His residues per tetramer are protonated in the transition at pH 5.7, suggesting that the activation of the M2 ion channel is closely linked with simultaneous protonation of all His37 residues in the tetramer [13].

3. Conformation, hydrogen bonding, and environment of Trp41

The structure and interaction of Trp41 have also been studied by UV Raman spectroscopy. The wavenumber of a Raman band (named W3) of Trp is known to be sensitive to the absolute value of the torsion angle, $|\chi^{2,1}|$, about the $C_2=C_3$–C_β–C_α linkage that connects the indole ring to the peptide main chain [27,30]. The W3 wavenumber of Trp41 decreases from 1553 to 1550 cm^{-1} on going from neutral to acidic pH [13], indicating a small change of $|\chi^{2,1}|$ from ~100° to ~90° [27]. Upon channel activation, the indole ring of Trp41 may be slightly reoriented or just turned over by a ~180° rotation about the C_3–C_β bond, though the latter conformational transition is less probable because flipping of the large indole ring would cause steric clash in the tetramer. Another Trp Raman band (W17) around 880 cm^{-1} is a marker of the strength of hydrogen bonding at the indole NH site [27,31]. The W17 Raman band of Trp41 appears at 878 cm^{-1} irrespective of pH, indicating that the indole ring of Trp41 is involved in a medium-strength hydrogen bond in both the active and inactive states of the channel [13]. It is probable that the indole ring of Trp41 is hydrogen bonded with a solvent water molecule throughout the channel activation and deactivation processes. The environment of the indole ring of Trp can be monitored by the intensity ratio of a Raman doublet (W7) at 1360/1340 cm^{-1} [27,32]. Analysis of the W7 doublet of Trp41 in M2-TMP has suggested highly hydrophobic environments in both the active and inactive tetrameric states, being consistent with the result of a fluorescence study that the environmental hydrophobicity of Trp41 increases with the formation of tetrameric bundles of the TM domain [33].

4. Cation–π interaction between His37 and Trp41

In conjunction with the protonation of His37 at pH 5.7, Trp41 exhibited an unusual change of UV resonance Raman intensity: an intensity *decrease* with 244-nm excitation and an intensity *increase* with 229-nm excitation [13]. The indole ring of Trp has strong absorption bands in the UV region and its UV resonance Raman intensity is a sensitive probe of structural factors that affect UV absorption [27]. Usually, a change in hydrogen bonding and/or hydrophobic interaction causes an increase or a decrease of both 229- and 244-nm Raman intensities of Trp. Accordingly, the unusual behavior of the Trp41 Raman intensity cannot be explained by a change in hydrogen bonding and/or environmental hydrophobicity. To reveal the interaction responsible for the Raman intensity change of Trp41, UV Raman spectra were investigated for a model compound containing two indole rings linked to the nitrogen atoms of diaza-18-crown-6 [13]. In the presence of K^+ ions, the crown ether ring traps a K^+ ion and the indole side arms sandwich the trapped cation at a cation–indole distance of ~3.3 Å [34]. An electrostatic attraction between the positive charge of the K^+ ion and the negative charge of π electrons of the indole ring induces the close contact of the K^+ ion and the indole ring [34]. The cation–π interaction in the crown ether compound produces an unusual change in UV Raman intensity of the indole ring, which is very similar to that observed for Trp41 upon protonation of His37 [13]. This coincidence of Raman intensity behavior strongly suggests that the indole ring of Trp41 is involved in a cation–π interaction with the imidazolium ring of His37 at acidic pH. A close proximity of the His37 and Trp41 side chains in the M2 tetramer has recently been suggested by a spin echo double resonance NMR study [35].

5. Model structure of the M2 ion channel

The TM domain of the M2 protein forms an α-helix in lipid bilayers as revealed by circular dichroism studies [11–13]. Since His37 and Trp41 are separated by three residues Leu^{38}-Ile-Leu^{40}, the side chains of His37 and Trp41 protrude on the same side of the TM helix with a separation of one helix pitch. Accordingly, there is a possibility that the cation–π interaction occurs between His37 and Trp41 within a single helix. However, model building studies using rotamer libraries for side chain conformations demonstrated that the His37 side chain could not be located near the Trp41 side chain on the same helix in any combinations of allowed side chain conformations [13,35]. A statistical analysis of the occurrence of His-Trp interactions revealed that it is energetically unfavorable for a His-x-x-x-Trp sequence to form a helix with an intrahelical short contact of the His and Trp side chains [36]. Therefore, the remaining possibility is that the His37-Trp41 cation–π interaction occurs between different TM helices. The closest His37-Trp41 pair may be achieved between TM helices in adjacent positions, but not in the diagonal positions, of the tetrameric bundle. A model for the tetrameric M2 ion channel in the active channel state is illustrated in Fig. 1a with special emphasis on the inter-helical His37-Trp41 cation–π interaction.

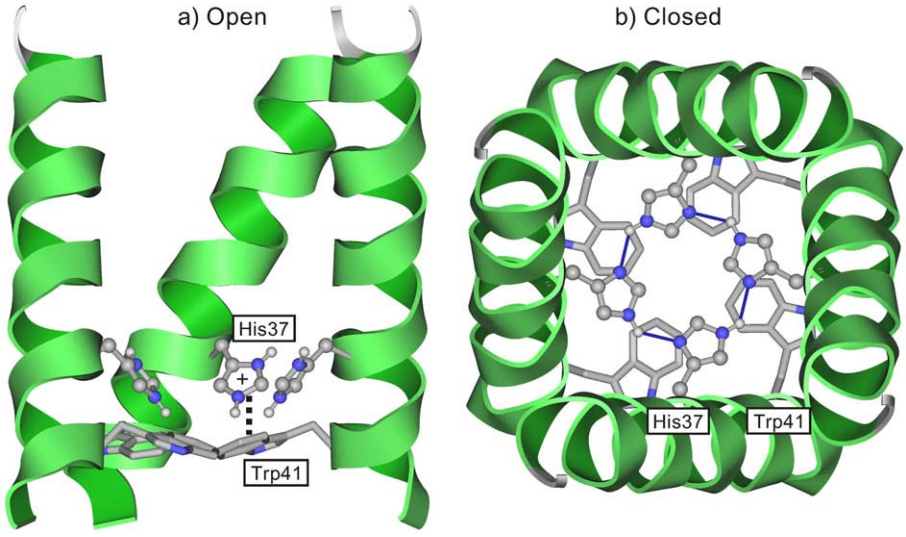

Fig. 1. Model of the M2 ion channel. The side chains of His37 and Trp41 are shown in ball-and-stick and stick formats, respectively. a: View from a direction perpendicular to the channel pore axis in the active (open) state. One of the four helices, nearest to the viewer, is omitted for clarity. The N-termini of the helices are located at the top. The broken line indicates the His37-Trp41 cation–π interaction between adjacent helices. The cationic imidazolium ring of His37 is attracted by the indole ring of Trp41, which also slightly reorients, and a pore for proton conductance is created. b: View from the N-terminal side of the channel axis in the inactive (closed) state. The thin lines indicate hydrogen bonds. The neutral imidazole rings of four His37 residues are linked by a network of hydrogen bonds and occlude the proton passage. The bulky indole ring of Trp41 also blocks the access of protons from the C-terminal side of the channel pore.

The cation–π interaction is increasingly recognized as one of the major non-covalent interactions that play a crucial role in determining the structures of proteins and in recognition of ligands by proteins [37–39]. Barnase, a microbial ribonuclease, provides an example of well-characterized cation–π interactions between His and Trp residues [40]. His18 of barnase is located at the C-terminal end of the first α-helix of the protein and its imidazole ring is in close contact with the indole ring of Trp94 located at the N-terminal end of a β-strand abutting on the first α-helix. The pK_a value of His18 has been determined to be 7.8, which is significantly higher than that (6.5) in the denatured protein devoid of specific inter-residue interactions [40]. Such elevation of pK_a is a common manifestation of cation–π interactions and is attributed to stabilization of the cationic imidazolium form of His by the negative charge of π-electrons of the Trp indole ring [37]. In the M2 ion channel, however, the pK_a value of His37 is decreased to 5.7, as opposed to an increase expected from the cation–π interaction with Trp41. The drop of the His37 pK_a value may be explained by assuming another interaction of His37 that stabilizes the neutral imidazole form more strongly than the cation–π interaction does the cationic imidazolium form. Energetically, a cation–π interaction is comparable to or stronger than a typical hydrogen bond [39], and the interaction opposing to the cation–π interaction must be stronger than a usual hydrogen bond.

His37 is isolated in a stretch of aliphatic non-polar residues in the primary structure of M2 protein, and residues other than His37 and Trp41 are unlikely to be involved in a strong interaction with His37 in a symmetrical bundle of four TM helices. As described above, the His37 side chain could not be located near the Trp41 side chain on the same helix [13,35]. Since the interaction with Trp41 on an adjacent helix contributes to the stabilization of the cationic form of His37, the interaction that stabilizes the neutral form is highly probable to involve another candidate of interaction partner, His37. The neutral imidazole ring has both a proton donor (protonated nitrogen) and a proton acceptor (non-protonated nitrogen). Thus, four His37 residues in the tetramer are capable of forming a network of cyclic hydrogen bonds, in which each His37 side chain acts as a proton donor at one nitrogen atom and as a proton acceptor at the other nitrogen atom (Fig. 1b). If this type of hydrogen bond network is established, the neutral form of His37 would be stabilized significantly, resulting in a drop of pK_a value of the imidazole ring. The His37 hydrogen bonding between adjacent helices may account for the analytical ultracentrifugation result that replacement of His37 with Ala or Phe impairs the tetramer formation of M2-TMP at neutral pH [41]. The networked His37 imidazole rings are likely to play an important role in blockage of a water-mediated proton transfer through the channel pore. The channel blocking properties of the neutral imidazole ring of His37 is consistent with the mutagenesis finding that the channel of the His37→Ala mutant does not close even at neutral pH [1]. Once the imidazole ring is protonated, the hydrogen bond network will be disrupted and the cationic imidazolium ring of His37 would move toward the indole ring of Trp41, making the channel pore open for proton passage as proposed previously by molecular dynamics simulations [42]. The His37-Trp41 cation–π interaction may be a prerequisite for the M2 ion channel to be fully opened. The hypothetical hydrogen bond network of His37 and the movement of the imidazole ring upon protonation await experimental verification.

In the wild-type M2 channel, the proton conductance is unidirectional and only the inward current from the N-terminal to the C-terminal side is passed through [9,43]. The Cu^{2+} ion is allowed to access His37 only from the N-terminal side [9,44]. In the Trp41→Ala mutant, however, the channel permits the outward current and Cu^{2+} ions become accessible to His37 from the C-terminal side [9]. These observations suggest that Trp41 acts as a gate that opens and closes the C-terminal

end of the channel pore [9]. Possibly, the bulky hydrophobic indole ring of Trp41 may block not only Cu^{2+} ions but also smaller protons (or hydronium ions) in the channel closed state. In the channel open state, the cation–π interaction between His37 and Trp41 may induce a slight reorientation of the Trp41 indole ring as suggested by Raman spectroscopy, leaving a small pore for proton passage.

The His-x-x-x-Trp motif is essential for formation of the pH-sensitive proton channel in the tetrameric M2 protein from influenza A virus. An analogous motif is also found in the amino acid sequence of the BM2 protein from influenza B virus. Very recently, Paterson et al. [45] have reported that the BM2 protein has properties expected for an integral membrane protein and is expressed at the surface of virus-infected cells with a peptide orientation analogous to that of the M2 protein. The putative TM domain of the BM2 protein spans residues 7–25 including a His-x-x-x-Trp motif (His^{19}-Phe-Met-Ala-Trp^{23}) in the C-terminal region. The location of the motif in the TM domain of BM2 also resembles that of the M2 protein. Presumably, the BM2 protein forms a channel, which conducts protons by using His-Trp interactions in a mechanism analogous to that of the M2 proton channel.

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