FEBS Letters 587 (2013) 2980–2983

FEBS Letters

journal homepage: www.FEBSLetters.org

The helical hairpin structure of the influenza fusion peptide can be seen on a hydrophobic moment map

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ARTICLE INFO

Article history:
Received 25 April 2013
Revised 26 June 2013
Accepted 27 June 2013
Available online 11 July 2013

Edited by Stuart Ferguson

Keywords:
Membrane fusion
Influenza virus
Hydrophobic moment
Amphiphilic helix
Sequence analysis

ABSTRACT

An assignment of the helical hairpin of the influenza fusion peptide has been made based on the hydrophobic moments, represented in a form of two-dimensional map. Such assignment holds for all serotypes, even for the cases of mutations altering the amino acid character. Similar results are obtained for the experimentally developed hydrophobicity scales, whose values reflect the transfer energies between aqueous and membrane environments. A distinct, however still structure-related hydrophobic map corresponds to a helical and contiguous HIV gp41 fp. The method may be used as a simple tool for sequence-based prediction of structures adopted by viral fusion peptides.

1. Introduction

Viruses having lipid bilayer envelopes enter into cells through fusion of viral and cellular membranes. This process is facilitated by viral fusion proteins which from one side are embedded in the viral lipid envelope, while the other fragment anchors in the endosomal membrane of a host cell [1,2]. The anchoring, usually amphiphilic fragment is named as fusion peptide (fp), although this term is not a precise definition [3]. Acidification of endosomes leads to large conformational changes of entire fusion proteins, what helps in bringing two membranes together and eventually in their fusion [1,2]. The drop of pH was also shown to affect the partitioning and fusogenic properties of various viral fusion peptides themselves, studied usually as short synthetic fragments.

During the cellular infection by influenza virus, membrane fusion process is mediated by hemagglutinin (HA) fusion peptide (HAfp), which serves as one of the best examples of enveloped virus cellular entry mechanisms [2]. The HAfp liquid-state NMR structure in detergent micelles was described as an amphiphilic helix with a kink in the middle, more pronounced at lower pH [4,5]. This helix-turn-helix motif was also confirmed in lipid environment by means of solid-state NMR [6]. Recent structures of the complete HAfp (i.e., HAfp 1–23), containing the conservative W21-Y22-G23 residues missing in previous studies, showed the existence of a tight helical hairpin structure observed even at pH 7.4 [7]. The middle G13 adopts the hairpin turn that links the two α-helices formed by G1–G12 and W14-G23, amphiphilic themselves separately (Figs. 1B, 2A). Despite the increasing number of HAfp structures, the mechanism of viral membrane fusion remains not fully understood, however it is likely the unique tight helical hairpin structure and its rearrangements play a pivotal role in this process.

Amphiphilic α-helices, although first observed in globular proteins, are the major structural elements involved in membrane-related phenomena. Their structure is characteristic because of an ordered spatial segregation of hydrophilic and hydrophobic residues along the long helical axis. Hydrophobic moment $\langle\mu_H\rangle$ (Eq. (1)), first introduced by Eisenberg, is the most commonly used parameter for quantitative analysis of amphiphilicity [8]. In this original seminal paper, $\langle\mu_H\rangle$ values were plotted against the average hydrophobicity $\langle H\rangle$, now referred as Eisenberg plot, and used for clustering of the three classes of helical fragments occurring in globular, transmembrane and 'surface' proteins [8]. Besides numerous applications in a form close to the one proposed originally, some adapted methodologies were further developed for subclassifications of amphiphilic α-helices [9]. Recently hydrophobicity and hydrophobic moments were used for an assignment of

Abbreviations: fp, fusion peptide; HAfp, hemagglutinin fusion peptide; $\langle\mu_H\rangle$, mean hydrophobic moment

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0014-5793/$36.00
http://dx.doi.org/10.1016/j.febslet.2013.06.054

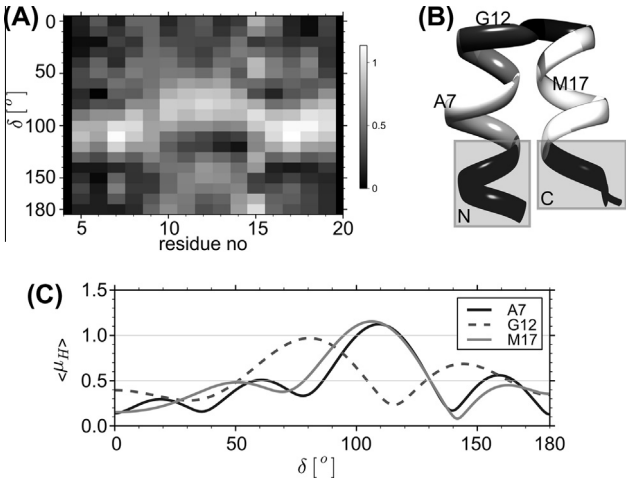

Fig. 1. (A) ⟨μH⟩ values plotted in gray scale as a function of the turn angle δ and the middle of amino acid window used for calculation. (B) Secondary structure of HAfp colored by the values of ⟨μH⟩ for 100° (rescaled for black as the minimum and white as the maximum value). Terminal amino acids not included for calculations due to a 9-amino acid window size are shaded with gray rectangles (PDB code: 2KXA). (C) Hydrophobic moment profiles for selected residues.

the transmembrane helices of *Escherichia coli* Complex I [10]. However ⟨μH⟩ values depend not only on the amphiphilicity, but also on parameters such as the helical turn angle and the hydrophobicity scale used (for a detailed review see Phoenix and Harris [11] and references therein). Therefore care must be taken during direct comparisons of ⟨μH⟩ values.

In this paper it has been observed that a helical hairpin structure of HAfp can be assigned based on its sequence from the hydrophobic moment map, depicting ⟨μH⟩ for two variables: the middle of amino acid window and the helical turn angle used for calculations. The applicatory potential of such approach was further exemplified for HIV gp41 fp, which contrary to HAfp shows a contiguous helical structure and a corresponding, distinct pattern on a hydrophobic moment map.

2. Materials and methods

The mean hydrophobic moments ⟨μH⟩ were calculated according to the original Eisenberg paper [8]:

$$\mu_H = \left(\left[\sum_{K=1}^{n} H_k sin(k\delta) \right]^2 + \left[\sum_{K=1}^{n} H_k cos(k\delta) \right]^2 \right)^{1/2} \quad (1)$$

$$< \mu_H >= \mu_H / n \quad (2)$$

where δ denotes the helical turn angle and H_k is the hydrophobicity of the residue k. The window of n = 9 residues was used in the main analysis, except for the comparison illustrated in Fig. S1. In all figures the ⟨μH⟩ values are given for the middle residue in the n-residue segment, thus they cannot be assigned for the first and the last four peptide residues (marked also with shaded rectangles on peptide structures in Figs. 1B and 3B).

The turn angle δ was changed in the range from 0° to 180° in steps of 10° for plotting the maps (Figs. 1A and 3A) and in steps of 0.5° for plotting the profiles (Figs. 1C and 3C). In the main analysis, Wimley and White whole residue octanol scale was used [12]. All calculations were performed in Python scripting language (www.python.org). Peptide structures were drawn with Chimera (www.cgl.ucsf.edu/chimera).

3. Results and discussion

3.1. Structure assignment of the influenza fusion peptide

To see whether the amphiphilic character of HAfp is reflected in the ⟨μH⟩ profiles, a hydrophobic moment map (see Section 2 for details) was drawn (Fig. 1A). It is noteworthy that, the maxima of ⟨μH⟩ were localized for the residues A7 and M17, lying in the central parts of the two helices assembling the hairpin structure. With $n = 9$ residue window, the positions of maxima corresponded to the regions 3–11 and 13–21, respectively, matching very well the structures of both, individual hairpin helices (Fig. 1B). Moreover, the overall ⟨μH⟩ profiles, both sequential and angular cross-sections, do not change when amino acid windows varied by length are used for its calculation (Fig. S1). The maxima are slightly shifted, however the assignment of N- and C-terminal regions still corresponds to the helical hairpin structure composed of two α-helical arms.

As discussed in the literature, calculation of ⟨μH⟩ for a wide range of angles may facilitate the detection of secondary structure elements [11]. It can be seen from Fig. 1A that the maxima for A7 and M7, plotted as a map cross-section in Fig. 1C, correspond to δ angles between 100° and 110°, close to an ideal α-helix, what agrees with the structure. Other local maxima are observed for

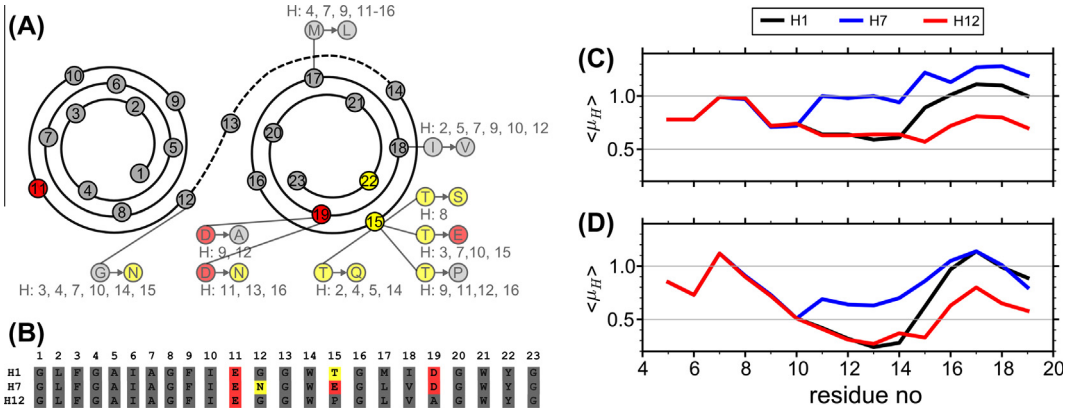

Fig. 2. (A) Helical wheels (wenxiang diagrams) for N- and C-terminal helical regions of HAfp (based on PDB code: 2KXA) and the mutations occurring in H1–H16 serotypes. Amino acids colored by type: gray-hydrophobic, polar-yellow, negatively charged-red. (B) Sequences of H1, H7 and H12 serotypes used for comparison with the same amino acid color coding. (C, D) Sequential hydrophobic moment cross-sections for fixed δ angles of 100° and 110°, respectively, which maximize ⟨μH⟩ for A7 and M17 (see Fig 1C). Profiles drawn for $n = 9$ amino acid window.