Biochimica et Biophysica Acta 1788 (2009) 2132–2141

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Membrane topology of gp41 and amyloid precursor protein: Interfering transmembrane interactions as potential targets for HIV and Alzheimer treatment

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ARTICLE INFO

Article history:
Received 1 May 2009
Received in revised form 29 June 2009
Accepted 13 July 2009
Available online 18 July 2009

Keywords:
gp41
Amyloid precursor protein
Transmembrane segment
Membrane
HIV
Alzheimer

ABSTRACT

The amyloid precursor protein (APP), that plays a critical role in the development of senile plaques in Alzheimer disease (AD), and the gp41 envelope protein of the human immunodeficiency virus (HIV), the causative agent of the acquired immunodeficiency syndrome (AIDS), are single-spanning type-1 transmembrane (TM) glycoproteins with the ability to form homo-oligomers. In this review we describe similarities, both in structural terms and sequence determinants of their TM and juxtamembrane regions. The TM domains are essential not only for anchoring the proteins in membranes but also have functional roles. Both TM segments contain GxxxG motifs that drive TM associations within the lipid bilayer. They also each possess similar sequence motifs, positioned at the membrane interface preceding their TM domains. These domains are known as cholesterol recognition/interaction amino acid consensus (CRAC) motif in gp41 and CRAC-like motif in APP. Moreover, in the cytoplasmic domain of both proteins other α-helical membranotropic regions with functional implications have been identified. Recent drug developments targeting both diseases are reviewed and the potential use of TM interaction modulators as therapeutic targets is discussed.

Contents

1. Introduction

Biological membranes are complex mixtures composed primarily of lipids and proteins. Although membrane proteins represent approximately one third of all proteins encoded in the human genome, and are involved in almost every aspect of cell biology and physiology, there is still little knowledge about how these proteins act and interact in biological membranes [1]. This is despite that more than half of currently marketed pharmaceuticals are targeting membrane proteins [2].

The vast majority of membrane proteins are anchored to cellular membranes through transmembrane (TM) domains that predominantly adopt an α-helical secondary structure [3]. Membrane-spanning α-helices, rather than serving merely as featureless hydrophobic stretches required for anchorage and facilitating insertion of proteins in membranes, have recognized functions well beyond

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doi:10.1016/j.bbamem.2009.07.008

these classical roles (for recent overviews see [4,5]). The organization and number of TM segments varies between membrane proteins, but it is generally believed that van der Waals interactions play an important role in the packing of TM domains. These interactions compensate for the lack of the hydrophobic effect that drives the folding of water-soluble proteins. Modulation of TM helix–helix interactions provides new exciting means to regulate the functions of membrane proteins. It is well established that homo- or hetero-dimerization, trimerization and other types of TM associations play important roles in different biological processes [5]. In the present review, we will discuss recent results on the structure, packing determinants and assembly of the TM domains of HIV gp41 and APP. These are both membrane proteins implicated in human diseases of paramount importance. The development of exogenous agents that recognize TM domains can be used for rational drug design [1,6], and by interfering with TM interactions new targeted therapeutics should be expected in the near future.

2. HIV envelope (Env) glycoproteins

Human immunodeficiency virus type-1 (HIV-1) is an enveloped virus that gains entry into target cells by mediating the fusion of viral and cellular membranes. Entry into cells is directed by the envelope (Env) glycoproteins, which are present on the surface of HIV-1 virions as trimers [7]. HIV-1 Env complex is synthesized as a type-1 TM gp160 precursor, which undergoes oligomerization, disulfide bond formation and extensive glycosylation, and is then post-translationally cleaved into the surface receptor binding subunit gp120 and the TM fusion protein gp41 [8], which remain non-covalently associated [9].

The full-length monomeric gp41 TM glycoprotein consists of three domains (Fig. 1A): an ectodomain (ECD), a TM domain and a large cytoplasmic domain (CTD). Several regions in the ECD are important for membrane fusion activity (see refs [10,11] for recent reviews): a highly conserved (glycine-rich) hydrophobic fusion peptide (FP), located at the extreme N-terminus; N- and C-terminal heptad repeat (HR) regions (NHR and CHR), connected by a glycosylated 30–40 residue disulfide-bonded loop; and a tryptophan-rich membrane-proximal ectodomain region (MPER). Binding of gp120 to the CD4 cellular receptor on the surface of target cells triggers a series of conformational changes in gp120 subunit that facilitate gp120 binding to a co-receptor, CXCR4 or CCR5, and the exposure of the hydrophobic gp41 fusion peptide. The dynamics of gp41 conformational changes triggering membrane fusion have been reviewed extensively [11–13]. Briefly, three gp41 NHR regions can adopt a parallel triple-stranded coiled-coil configuration that enables penetration of the gp41 fusion peptide into the membrane of the target cell. Subsequent refolding of gp41 heptad repeat (HR) regions into a six-helix bundle structure (trimer-of-hairpins) forces the juxtaposition of the viral and cell membranes, promoting their fusion [14]. Recently, the C-terminal boundary of this six-helix bundle fusion conformation in an ongoing dynamic fusion process has been demonstrated [15]. At present, it is thought that the structural rearrangements in the gp41 TM glycoprotein are crucial for the membrane fusion process and viral entry [16].

3. gp41 membranotropic sequences

Although gp41 six-helix bundle formation is the main driving force for the fusion process, other gp41 regions in the ECD may regulate fusion activity in numerous ways. The role of the N-terminal fusion peptide region and its implication in membrane destabilization and fusogenic activity has been analyzed in recent reviews [10,11]. The membrane conformation of the fusion peptide (α-helical/β-strand/disordered) deduced from chemically synthesized FPs in model membranes is controversial [17–19], probably due to an effect of microenvironment composition dictating the adopted conformation [20]. In this context, gp41 FP, which is unstructured in solution, adopts an α-helical structure in micelles [21,22], inserting its N-terminal residues in an α-helical conformation and presenting a flexible hinge reminiscent of the kinked structure proposed for several N-terminal fusion peptides [23–26]. Structural plasticity of gp41 FP has been also observed depending on peptide concentration. When bound to lipid bilayers at low concentration gp41 FP is largely α-helical, however, at higher protein/lipid ratios the domain is partially converted to form β-structures [19]. A ^{13}C FTIR study have demonstrated that this peptide adopts an intermolecular parallel β-sheet structure in membranes when stabilized by the adjacent N-terminal heptad repeat [27]. Recent 2D correlation spectra and distance measurements from solid-state NMR-spectroscopy in cholesterol-containing host-cell-like membranes indicated that the fusion region had predominantly a β-strand conformation [28], with 50–60% population of

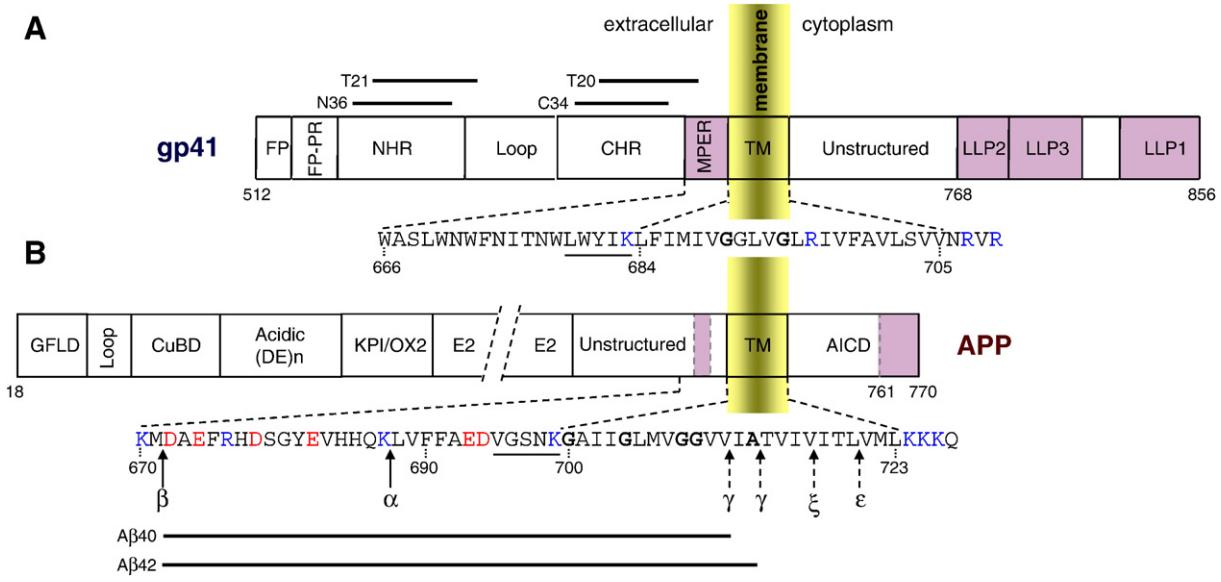

Fig. 1. Schematic representation of HIV-1 gp41 (A) and APP (B). TM domain and membranotropic sequences in each protein are depicted darker. TM and juxtamembrane regions are enlarged and for APP the sequence involved in processing is shown with the major sites of cleavage by α-, β-, and γ-secretases highlighted. Dashed arrows indicate APP intramembrane cleavage sites. The CRAC motifs are underlined. TM glycines and alanine residues involved in GxxxG/A motifs are shown in bold. Locations of gp41 inhibitor peptides and Aβ 40/42 peptides are depicted with dark lines. Numbering refers to HIV gp160 precursor, BH10 isolate (A) and human APP770 isoform (B). See text for details.