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Review

Peptide entry inhibitors of enveloped viruses: The importance of interfacial hydrophobicity ☆

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ABSTRACT

There are many peptides known that inhibit the entry of enveloped viruses into cells, including one peptide that is successfully being used in the clinic as a drug. In this review, we discuss the discovery, antiviral activity and mechanism of action of such peptides. While peptide entry inhibitors have been discovered by a wide variety of approaches (structure-based, accidental, intentional, rational and brute force) we show here that they share a common physical chemical property: they are at least somewhat hydrophobic and/or amphipathic and have a propensity to interact with membrane interfaces. We propose that this propensity drives a shared mechanism of action for many peptide entry inhibitors, involving direct interactions with viral and cellular membranes, as well as interactions with the complex hydrophobic protein/lipid interfaces that are exposed, at least transiently, during virus–cell fusion. By interacting simultaneously with the membrane interfaces and other critical hydrophobic surfaces, we hypothesize that peptide entry inhibitors can act by changing the physical chemistry of the membranes, and the fusion protein interfaces bridging them, and by doing so interfere with the fusion of cellular and viral membranes. Based on this idea, we propose that an approach that focuses on the interfacial hydrophobicity of putative entry inhibitors could lead to the efficient discovery of novel, broad-spectrum viral entry inhibitors. This article is part of a Special Issue entitled: Interfacially Active Peptides and Proteins. Guest Editors: William C. Wimley and Kalina Hristova.

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1. Introduction

Enveloped viruses are an ancient and ubiquitous class of human pathogen with infection rates and mortality rates that are often expressed as measurable percentages of the entire human population [1,2]. This class includes many well-known viruses such as influenza, human immunodeficiency, hepatitis C, small pox, chicken pox, yellow fever, herpes, measles and many more. It also includes tropical pathogens of significant and growing global public health concern (e.g. dengue, lassa and chikungunya viruses), viruses that have recently elicited fears of novel and deadly global pandemics (e.g. avian influenza, SARS and MERS viruses) and viruses with significant biothreat potential (e.g. ebola, hantaviruses, Rift Valley fever virus, and most of the viruses mentioned above). In this review, we discuss the discovery, development, characterization and mechanism of action of peptides that inhibit entry of enveloped viruses into host cells.

Over the past few decades peptides have steadily gained importance in drug design and delivery. Increasingly, focus is shifting toward the development and refinement of techniques for identifying synthetic peptides as drug candidates. Bioactive peptides have been discovered by the use of naturally occurring peptides, through rational engineering, through high-throughput screening, or by structure-based design using sequences from known regions of proteins [3]. These factors are responsible for the emergence of peptides as a growing market in the pharmaceutical industry. Currently there are about 100 peptide based drugs on the market [4], constituting about 10% of the entire drug market [5]. As we describe below, one effective peptide entry inhibitor of an enveloped virus is approved for use in humans [6] and more are in clinical trials [7,8]. Many other peptide entry inhibitors of enveloped viruses have been described in the scientific literature. In this review we discuss the surprising observation that the majority of known peptide entry inhibitors, which have been discovered by an extraordinary diversity of approaches, share a common physical chemistry: they are somewhat hydrophobic and amphipathic with a propensity for binding to lipid membranes. We hypothesize that their shared physical chemistry could result in a shared mechanism of action; one that enables a generic approach to the discovery of broad-spectrum peptide entry inhibitors for enveloped viruses.

2. Enveloped viruses

2.1. Entry of enveloped viruses into cells

The genomes of all enveloped viruses enter cells through a sequential, multistep process that requires i) virus binding to cell surface receptors and ii) fusion of the viral membrane with a cellular membrane [9]. These essential steps occur by an incompletely understood process in which viral fusion proteins (i.e. envelope glycoproteins, spike proteins, see Fig. 1) mediate a temporally and spatially coordinated close contact between the cellular and viral membranes while simultaneously perturbing the continuity of the membranes with hydrophobic segments. For most enveloped virus families these events are triggered by endocytosis of surface-bound virus and subsequent endosomal acidification. For a few viruses, such as HIV, fusion is triggered by conformational changes that occur upon binding to cell surface receptors and co-receptors and is not entirely dependent on endosomal acidification.

The fusion proteins of enveloped viruses can be grouped into several distinct classes based on the protein secondary and tertiary structure. Class I fusion proteins are predominantly α-helical trimers that fold into an elongated 6-helix bundle [10–12]. This class includes the well-known "spike" proteins of influenza (hemagglutinin) and HIV (gp160). Class II fusion proteins are defined by their elongated, multi-domain, β-sheet rich structure and are found, for example, in flaviviruses. Class II fusion proteins are dimeric in the pre-fusion configuration, but transition to a trimeric state during low pH-induced fusion [13]. Class III fusion proteins have mixed α/β structure and are found in, for example, the herpes viruses [14].

Despite the structural diversity of their fusion proteins, the entry mechanisms of enveloped viruses share critical biological activities and structural signatures (Fig. 2). For all classes, cell surface binding is driven by a specific chain or domain in a fusion protein or protein complex that is distinct from the domain/chain that drives fusion of the viral envelope with the cellular membrane. Membrane fusion is driven by large conformational rearrangements of the fusion protein that expose a hydrophobic fusion peptide or fusion loop while also simultaneously presenting other hydrophobic sequences of the fusion protein to catalyze membrane fusion [15–17].

In Fig. 2 we show schematic structures of representative Class I, Class II and Class III viral fusion proteins in the pre- and post-fusion states. In addition to the hydrophobic fusion peptide (which is a terminal peptide in Class I proteins, an internal loop in Class II proteins and a pair of fusion loops in Class III fusion proteins) viral fusion proteins also have other hydrophobic sequences that likely interact with membranes and contribute to the fusion process. For example, many have a conserved, hydrophobic, aromatic-rich domain adjacent to the membrane-spanning helix domain. In HIV, this so called "membrane proximal ectodomain region" or MPER is highly conserved and is critical for function [6]. The MPER sequence of HIV gp41 is also the epitope for one of the few broadly neutralizing antibodies against HIV [18]. MPER is very hydrophobic,

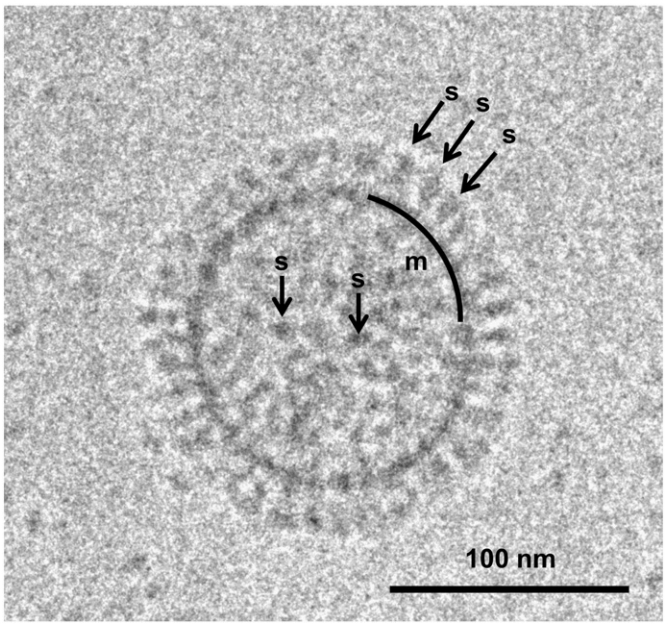

Fig. 1. Cryo-transmission electron microscopy (CryoTEM) image of an influenza virus particle, showing its classical enveloped virus architecture. The lipid bilayer membrane is bounded on the inner surface by electron-dense matrix proteins (m). The membrane is packed with 16 nm long spikes(s) made of the Class I fusion protein hemagglutinin, here in the pre-fusion (neutral pH) state.