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Antivirals acting on viral envelopes via biophysical mechanisms of action

Sietske Speerstra[a], Alexey A. Chistov[b], Gleb V. Proskurin[b], Andrey V. Aralov[b], Egor A. Ulashchik[c], Philipp P. Streshnev[b], Vadim V. Shmanai[c], Vladimir A. Korshun[b], Luis M. Schang[a,d,*]

[a] Department of Biochemistry, University of Alberta, Edmonton, AB T6G 2S2, Canada
[b] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Moscow, 117997, Russia
[c] Institute of Physico-Organic Chemistry, Minsk, 220072, Belarus
[d] Baker Institute for Animal Health, Cornell University, Ithaca, NY 14853, United States

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ABSTRACT

Most antivirals target viral proteins and are specific for only one virus, or viral type. Whereas viral proteins are encoded in the plastic viral genome, virion lipids are not and their rearrangements during fusion are conserved among otherwise unrelated enveloped viruses. Antivirals that inhibit these lipid rearrangements could thus pose a high barrier to resistance and have broad-spectrum activity.

Fusion occurs through a hemifusion stalk in which only the outer leaflets are fused and thus curved with a smaller radius for the polar heads than for the hydrophobic tails (negative curvature). Outer leaflets enriched in phospholipids with head groups of larger cross sections than their lipid tails ("inverted cone") disfavor negative curvature, inhibiting fusion. The rigid amphipathic fusion inhibitors (RAFIs) are synthetic compounds of inverted cone molecular geometry. They inhibit infectivity of otherwise unrelated enveloped viruses. The leading RAFI, aUY11, has an ethynyl-perylene hydrophobic and an uracil-arabinose polar moiety. aUY11 intercalates in viral envelopes and inhibits virion-to-cell fusion of a broad spectrum of otherwise unrelated enveloped viruses. Previous studies showed that amphipathicity, rigidity, and inverted cone molecular geometry were required. We propose that the inverted cone molecular geometry of the RAFIs increases the energy barrier for the hemifusion stalk, inhibiting fusion. Then, chemically distinct compounds with similar amphipathicity, rigidity, and inverted cone shape would have similar antiviral potencies, regardless of specific chemical groups. Alternatively, the perylene group exposed to visible light may induce viral lipid peroxidation. Then, the perylene group and absorbance at visible spectrum would be required. We now evaluated twenty-five chemically distinct RAFIs. The perylene moiety and absorption at visible spectrum were not required, but a minimum length of the hydrophobic moiety was, 10.3 Å. The arabino moiety could be modified or replaced by other groups. Cytidine was not tolerated. Bilayer intercalation was required but not sufficient. The vast majority of RAFIs had no overt cytotoxicity (CC_{50} > 20 μM; TI > 250–1200). Carbonyl or butylamide substitutions for arabino, or cytidine replacement for uracil, increased cytotoxicity. Cytotoxicity was mainly determined by the polar moiety and there was no correlation between antiviral and cytostatic activities.

The definition of the effects of shape and chemical groups of the RAFIs opens the possibility to the rational design of lipid-acting antivirals active against a broad spectrum of enveloped viruses.

1. Introduction

Inhibition of viral entry is an attractive antiviral target (De Clercq and Li, 2016; Colpitts and Baumert, 2016; Lee et al., 2017; Mitchell et al., 2017; Pietschmann, 2017; Xiao et al., 2014; Yamauchi and Helenius, 2013). Entry inhibitors prevent infection altogether, before any cell damage can occur, and need not to be transported across membranes. Only three entry inhibitors are FDA-approved, however (De Clercq and Li, 2016): enfuvirtide, which acts on HIV gp41 (Kilby et al., 1998), maraviroc, which targets the HIV-1 CCR5 co-receptor (Dorr et al., 2005; Fätkenheuer et al., 2005), and palivizumab, an antibody against respiratory syncytial virus (RSV). They act on viral proteins and are thus specific for only one virus, or virus type (maraviroc, Raymond et al., 2015), and prone to select for resistance (Adams et al., 2010; Greenberg and Cammack, 2004; Ratcliff et al., 2013; Waters et al., 2008).

Most viruses attach to surface glycans and envelope-to-cell membrane fusion is conserved among all enveloped viruses. Compounds that

* Corresponding author. Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, United States.
E-mail address: lms428@cornell.edu (L.M. Schang).

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target such steps could have broad spectrum antiviral activity (Badani et al., 2014; Bobardt et al., 2008; Chamoun et al., 2012; Cheng et al., 2008; Colpitts and Schang, 2014; Edinger et al., 2014). They are also likely to present high selection barriers and could be active against only partially characterized emerging viruses.

Many important human pathogens are enveloped viruses, including influenza A - (IAV), human immunodeficiency - (HIV), or herpes simplex -virus (HSV) −1 and −2. For infection to occur, envelopes fuse with cellular membranes (Harrison, 2008) by merging two lipid bilayers through an intermediate structure, the hemifusion stalk (Aeffner et al., 2012; Chernomordik and Kozlov, 2005; Chlanda et al., 2016; Markosyan et al., 2000; Melikyan et al., 1995; Xu et al., 2005). In this stack, the outer leaflets are already fused while the inner leaflets are not (Chernomordik and Kozlov, 2005; Chernomordik et al., 1998). The outer leaflets are thus bent with negative curvature, in which the radius of the polar heads is smaller than that of the hydrophobic tails (Aeffner et al., 2012; Campelo et al., 2008; Chang and Jackson, 2015; Cohen and Melikyan, 2004; McMahon and Gallop, 2005; Stachowiak et al., 2013). The lipid rearrangements required for bending the leaflets pose one of the fusion energy barriers.

Lipids with head groups of larger cross sections than those of their lipid tails have inverted cone shapes. Outer leaflets enriched in these phospholipids tend to adopt positive curvatures, inhibiting the negative curvature required for fusion (Chernomordik and Kozlov, 2005; Chernomordik et al., 1995a, 1985; Gaudin, 2000; Gunther Ausborn et al., 1995; Vogel et al., 1993; Yeagle et al., 1994). Although such phospholipids have antiviral properties (Chernomordik et al., 1997; Gaudin, 2000), they are not suitable as antivirals; they are rapidly metabolized and often toxic.

The rigid amphipathic fusion inhibitors (RAFIs) are synthetic amphipathic molecules with polar moieties of larger cross sections than those of their rigid hydrophobic moieties (inverted cones) (Fig. 1). The first RAFIs, aUY11 and dUY11, have deoxyribose or arabinose-uridine polar moieties and ethynylperylene rigid hydrophobic moieties (Colpitts et al., 2013; St Vincent et al., 2010). They inhibit the infectivity of unrelated enveloped viruses, including IAV, HSV-1 and -2, hepatitis C virus (HCV), and others, at nanomolar concentrations. The RAFIs have no effects on the non-enveloped poliovirus, reovirus or adenovirus (Colpitts et al., 2013; St Vincent et al., 2010).

The RAFIs intercalate in hydrophobic environments of enveloped virions or protein-free liposomes (Colpitts et al., 2013; St Vincent et al., 2010), without affecting membrane fluidity (Colpitts et al., 2013). They inhibit virion-cell lipid mixing at similar concentrations as they inhibit infectivity of otherwise unrelated viruses (Colpitts et al., 2013; St Vincent et al., 2010). They also inhibit liposome-cell lipid mixing, indicating that they act independently of viral proteins (Colpitts et al., 2013). They inhibit transitions from lamellar to inverted hexagonal phases in bilayer stacks (Colpitts et al., 2013; St Vincent et al., 2010), transition which involves negative curvature intermediates. We proposed that the RAFIs target lipids to inhibit the formation of the negative curvature required for fusion (Colpitts et al., 2013; St Vincent et al., 2010). They have alternatively been proposed to trigger light induced phospholipid peroxidation (Vigant et al., 2014). Absorption in the visible spectra (Orlov et al., 2016) and the perylene moiety (Vigant et al., 2014) were proposed required to generate singlet oxygen species, which would peroxidise envelope lipids. This envelope lipid peroxidation would in turn increase the rigidity of the envelope (Vigant et al., 2014). A similar mechanism has been proposed for hypericin and LJ001 (Lenard et al., 1993; Vigant et al., 2014).

Membrane fusion is required for many cellular processes (Martens and McMahon, 2008). Intracellular fusions are mediated by N-ethylmaleimide-sensitive factor (NSF)-attachment protein receptor (SNARE) (Chen and Scheller, 2001). The SNAREs in the two fusing bilayers zipper progressively from their N-termini, bringing the membranes in close apposition. Post-fusion SNARE complexes are disassembled by NSF, consuming ATP (Söllner, 2003). Many other steps in cellular

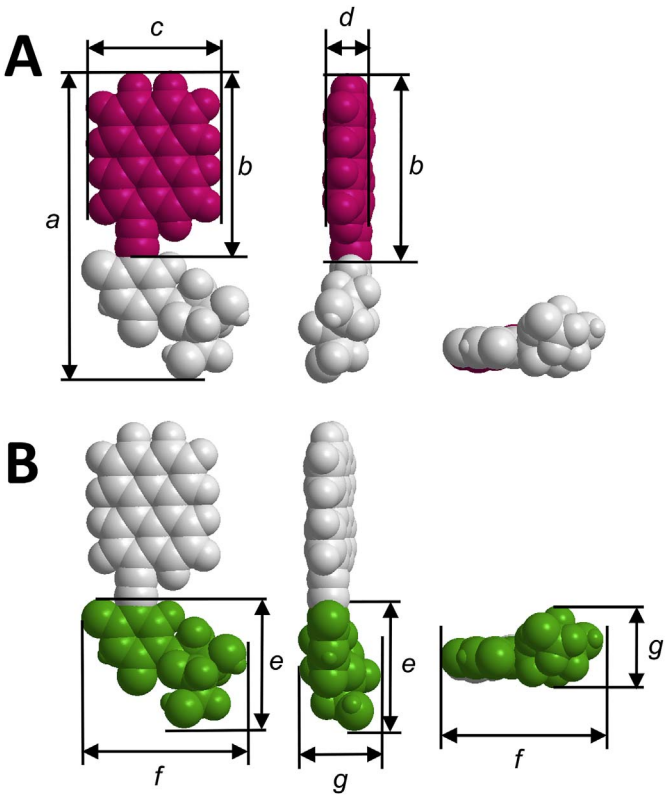

Fig. 1. Space filling structures of the RAFIs. Space filling structure of the RAFI aUY11 in three orthogonal perspectives, indicating dimensions of the hydrophobic (**A**, pink) or polar (**B**, green) moiety. The cross sections of the polar moiety (dimensions *f* and *g*) are larger than those of the hydrophobic moieties (dimensions *c* and *d*), giving the inverted cone shape. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

membrane fusion also use NTP hydrolysis. Rab GTPases enhance SNARE-mediated fusion efficiency (Lommer et al., 2009; Murray et al., 2016). GTP provides energy for atlastin-mediated fusions between endoplasmic reticulum membranes (Winsor et al., 2017). Dynamin uses GTP to induce membrane rearrangements, and clathrin cages and coat protein (COP) complexes modulate membrane curvatures (Chernomordik and Kozlov, 2003; Zimmerberg and Kozlov, 2006).

The RAFIs intercalate in cellular membranes but have no toxic effects (therapeutic index (TI - CC_{50}/EC_{50}) > 935, aUY11; > 3000 dUY11) (Colpitts et al., 2013; St Vincent et al., 2010). The mechanisms for this specificity for virion-to-cell over cellular fusions are unknown, but two models have been proposed based on the virions being metabolically inert (Colpitts et al., 2013; St Vincent et al., 2010; Vigant et al., 2014). The RAFIs may inhibit the fusion of enveloped viruses by increasing the hemifusion stalk energy barrier (Colpitts et al., 2013; St Vincent et al., 2010), whereas cells use metabolic energy to bring membranes together, remodel lipids and change membrane curvatures, overcoming the higher fusion energy barrier. The RAFIs would then inhibit virion-to-cell fusion based on their physical properties such as shape, rigidity and amphipathicity, rather than on chemical properties, such as reactive functional groups. Then, chemically distinct molecules with similar shape, rigidity, and amphipathicities should also inhibit infectivity, with no cytotoxicity and regardless of specific chemical groups. We tested the activities of twenty-five RAFIs with chemically distinct moieties against one model envelope virus, HSV-1.