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Disruption of the interactions between the subunits of herpesvirus DNA polymerases as a novel antiviral strategy

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ABSTRACT

Most biological processes depend on the co-ordinated formation of protein–protein interactions. Besides their importance for virus replication, several interactions between virus proteins have been proposed as attractive targets for antiviral drug discovery, as the exquisite specificity of such cognate interactions affords the possibility of interfering with them in a highly specific and effective manner. There is a considerable need for new drugs active against herpesviruses, since available agents, most of which target the polymerisation activity of the virus DNA polymerase, are limited by pharmacokinetic issues, toxicity and antiviral resistance. A potential novel target for anti-herpesvirus drugs is the interaction between the two subunits of the virus DNA polymerase. This review focuses on recent developments using peptides and small molecules to inhibit protein–protein interactions between herpesvirus DNA polymerase subunits.

Keywords DNA polymerase, drug discovery, herpesviruses, polymerisation interactions, protein–protein interactions, review

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INTRODUCTION

Eight herpesviruses are known to infect humans, and several of these viruses are important human pathogens [1]. These viruses cause a wide variety of diseases. Herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) cause herpes labialis and genitalis, keratoconjunctivitis and encephalitis; human cytomegalovirus (HCMV) is responsible for a variety of severe diseases in immunocompromised patients, including pneumonia, gastrointestinal disease and retinitis in transplant recipients and in AIDS patients, and is also a major cause of congenital defects in newborn children; varicella-zoster virus (VZV) is the causative agent of chicken pox following primary infection, but can reoccur in adults as herpes zoster (shingles); other members of the family include Epstein-Barr virus (EBV) and human herpesvirus 6, 7 and 8 (HHV-6, HHV-7 and HHV-8). Herpesvirus infections are increasing because of the growing number of immunocompromised individuals, i.e., transplant recipients and AIDS patients.

Antiviral agents licensed currently for the treatment of herpesvirus infections include acyclovir and derivatives, ganciclovir, foscarnet and cidofovir, all of which inhibit herpesvirus DNA polymerases [2]. Acyclovir, ganciclovir and cidofovir are nucleoside analogues which function as DNA chain terminators, whereas foscarnet inhibits virus DNA polymerase through binding to its pyrophosphate binding site. However, some of these antiviral agents, e.g., ganciclovir and foscarnet, can produce toxic side-effects. In addition, the emergence of virus strains resistant to commonly used anti-herpesvirus drugs is a growing problem, particularly in immunocompromised patients [3]. Therefore, there is still a great demand for the discovery of new, more effective and specific anti-herpesvirus agents.

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A novel strategy to inhibit virus replication is based on the disruption of virus protein–protein complexes by peptides or peptidomimetic compounds that mimic part of the interaction between subunits [4]. Many enzymes act as oligomer complexes, so inhibitors may act by preventing the formation of the active holoenzyme. Indeed, the first example in the literature of successful disruption of a protein–protein interaction involved an enzyme, the HSV-1 ribonucleotide reductase (RR). HSV-1 RR is a tetramer consisting of two large R1 subunits and two small R2 subunits [5]. In 1986, two independent groups of researchers reported that the synthetic nonapeptide YAGAVVNDL, corresponding to the C-terminus of the small subunit R2, could inhibit HSV-1 RR activity specifically by disrupting the interaction between the subunits [6,7]. Moreover, when the YAGAVVNDL peptide was linked to a protein carrier, the B subunit of *Escherichia coli* heat-labile enterotoxin, the resulting fusion protein inhibited virus replication and RR activity in HSV-1-infected cells specifically [8], thereby providing direct evidence of the antiviral efficacy of the YAGAVVNDL peptide in a cellular system. Thus, the YAGAVVNDL peptide became the first example of a new class of inhibitors that act by dissociating the subunits of multimeric enzymes.

Following these pioneering studies, a number of other peptides have been identified which disrupt protein–protein interactions between the subunits of other virus enzymes, e.g., the herpesvirus DNA polymerases. Replicative DNA polymerases generally function as multiprotein complexes, including a catalytic subunit and one or more accessory proteins that modify the properties of the core polypeptide. Analogously, it has been shown that a two-subunit DNA polymerase is a common theme among members of the Herpesviridae family, as the virus enzyme is composed of a catalytic subunit and an accessory protein, which is proposed to act as a processivity factor (Fig. 1). The best studied herpesvirus DNA polymerase is the HSV-1 polymerase, which is a heterodimer composed of two proteins, UL30 and UL42 [9]; in HSV-2, the infected cell-specific peptide (ICSP) 34,35 has been shown to be the counterpart of UL42 [10]. VZV polymerase interacts with a DNA-binding protein (encoded by gene 16) which also shows sequence similarity with UL42 [11]; in equine herpesvirus-1, proteins homologous to UL30 and

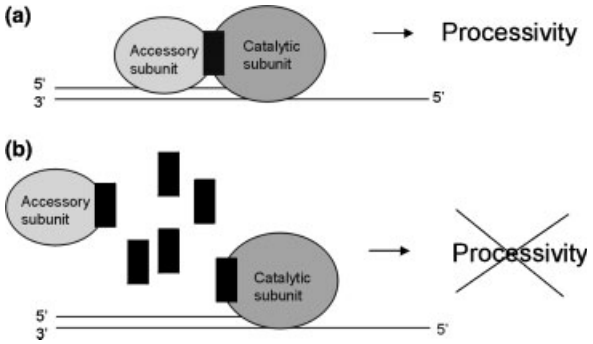

Fig. 1. Disruption of the herpesvirus DNA polymerase complex. (a) The DNA polymerase of herpesviruses is composed of a catalytic subunit, which possesses basal activity, and an accessory protein. By interacting with the catalytic subunit, the accessory protein stimulates the processivity of the enzyme. In both the HSV-1 and the HCMV DNA polymerase, the region of the catalytic subunit responsible for binding to the accessory protein has been localised to the C-terminal region (here represented as a rectangle). (b) Peptides corresponding to the C-terminal region of the catalytic subunit or small molecules mimicking the side-chain of residues crucial for subunit interaction are capable of disrupting the DNA polymerase complex, thus inhibiting the processivity of the virus enzyme.

UL42 are encoded by ORF30 and ORF18, respectively [12]. The HCMV DNA polymerase also consists of two proteins, UL54 and UL44 [13]. Other examples include the two subunits of pseudorabies virus DNA polymerase [14], the BALF5/BMRF1 complex of EBV [15], the catalytic subunit (Pol6) and the accessory protein (p41) of HHV-6 DNA polymerase [16,17], and the Pol8 and PF-8 subunits of HHV-8 DNA polymerase [18].

During the past few years, research interest has focused on the development of new anti-herpesvirus inhibitors which act by disrupting the interaction between the subunits of herpesvirus DNA polymerases, i.e., HSV-1 and HCMV DNA polymerase. Efforts have been aimed at characterising protein–protein interactions between the enzyme subunits, and at identifying peptides and small molecules that mimic either face of the subunit interaction, and which are therefore able to disrupt the virus protein complexes.

Herpes simplex virus DNA polymerase

The HSV-1 DNA polymerase is a heterodimer composed of a catalytic subunit, Pol or UL30, and an accessory protein, UL42, which stimulates the