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Review Article

Griffithsin; A Potential Therapeutic Agent for SARS-CoV-2

Saba Siddiqui[1] and Armin Ahmed[2]*

[1]*Associate Professor and Head, Department of Agriculture, Integral Institute of Agricultural science and technology (IIAST), Lucknow, India*

[2]*Associate Professor, Department of Critical Care Medicine, King George Medical University, Lucknow, India*

***Corresponding Author:** Armin Ahmed, Associate Professor, Department of Critical Care Medicine, King George Medical University, Lucknow, India.

E-mail: drarminahmed@gmail.com

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Abstract

Griffithsin is a red algal protein with promising broad-spectrum antiviral action. It exists as a homodimer, and every monomer has three monosaccharide binding sites arranged as an equatorial triangle. It acts in the early part of the viral disease by hampering viral entry inside the cell. Envelop protein of many viruses shows rich glycosylation with mannose which binds with monosaccharide binding sites of Griffithsin in a multivalent binding fashion, thus bringing changes that interfere with the host cell invasion. Previous *in-vitro* and *in-vivo* studies on SARS-CoV and MERS-CoV have shown excellent inhibitory action on these viruses with no or minimal toxicity. It has also been studied for its synergistic antiviral action with Carrageenan (another algal derivative) and EK1 (a pan-coronavirus fusion inhibitor). There is growing interest in Griffithsin as a prophylactic and therapeutic option for the current COVID 19 pandemic due to its potent action, topical application causing inhibition of viral entry, safety profile, chemical stability, easy large-scale production, and synergistic action with other antiviral agents.

Keywords: Griffithsin; Coronaviruses; SARS-CoV; SARS-CoV-2

Introduction

Since the prehistoric era, humanity has taken refuge in nature to find a cure for several diseases and ailments. Reports indicate that more than sixty percent of the approved anti-infective and anti-tumor drugs owe their origin in terms of structure from the natural molecules [1]. National Cancer Institute (NCI) laboratory, while working on anti-HIV (human immunodeficiency virus) peptide and protein elucidation, isolated several potential microbicides from different natural resources. In continuation with the discoveries from the extracts of cyanobacteria (Cyanovirin-N and Scytoynin), a unique anti-HIV protein was discovered from an aqueous extract of *Griffithsia* named red alga found in marine waters. The protein was named Griffithsin [2]. Aqueous extracts of *Griffithsia*, which were collected from waters of New Zealand, exhibited potent cytoprotective activity against the cytopathogenicity of HIV-1. Before the discovery of this property, *Griffithsia* was already being exploited for other metabolites, photosynthetic pigments, phycoerythrin proteins, and polysaccharides [3]. Other organic constituents from *Griffithsia* have never shown antiviral properties but Griffithsin represents a unique potential microbicidal candidate against many enveloped viruses. It is a relatively stable molecule with good feasibility for large-scale production. It can be used in combination with other antiviral agents for synergistic and broad-spectrum antiviral action.

The ongoing COVID-19 pandemic due to recently discovered coronavirus SARS-CoV-2 has caused 5,570,163 deaths across the globe by January 2022 [4]. There is an urgent need to find a safe and cost-effective antiviral drug that can be made readily available

for the masses. The relentless search for effective COVID 19 treatments has led to the discovery of new medicines and the repurposing of already existing antivirals.

The current article evaluates the notion that this natural product, Griffithsin, which can fight viral infections such as HIV, SARS (severe acute respiratory syndrome), MERS (middle east respiratory syndrome), and Ebola viral disease, can also act as a potential therapeutic agent against SARS-CoV-2.

Griffithsin - A unique lectin

Griffithsin belongs to a group of sugar-binding proteins called lectins which are ubiquitously found in different microbes and animals. These are also upcoming antiviral agents with therapeutic potential. A few recently discovered lectins are Bananalectin, Cyanovirin, Scytovirin, Microvirin, and Griffithsin [5-9]. Among these, Griffithsin has been found to be the most promising antiviral compound. It is unique because it has not been found to have any homology with the proteins reported to date. It is reported to contain an unusual amino acid (151.05 Da) at position 31.

Griffithsin has a special predilection for binding with mannose. This helps it to break the outer cover of the enveloped viruses with mannose-rich glycosylation. Its sequence was determined through a combination of N-terminal Edman degradation of the intact protein and N-terminal sequencing of peptide fragments obtained from endopeptidase and cyanogen bromide treatments [5].

Griffithsin is present as a homodimer, with each subunit made of 121 amino acids. Each of the subunits of homodimer shows internal threefold symmetry. The three binding sites of the Griffithsin monomer appear to form a triangle and can bind three monosaccharides [10].

Comparison with other plant lectins

Griffithsin appears to be unparalleled compared to other similar lectins because of the b-hairpin conformation, which consists of Gly66 and Asp67. Studies of crystal structures and modeling indicate that Griffithsin achieves high affinity with large carbohydrates via multivalent bonding. This occurs by the utilization of multiple monosaccharide-binding specific sites. These findings contrast with other lectins like *Jacalin, Maclura pomifera* agglutinin, and *Artocarpus hirsuta* lectin, which contain two separate chains, with a break between chains B and A [11].

It is also different from other contemporary antiviral proteins obtained from cyanobacteria (scytovirin or cyanovirin) which achieve their affinity for carbohydrates through an extended binding site.

Griffithsin - A broad-spectrum antiviral

The life cycle of any virus begins with entering the cell of a susceptible host, and the existence of host cell receptors usually determines its target ability. A diverse array of glycoproteins has evolved in enveloped viruses with different association capabilities with human receptors. Many antiviral drugs and vaccines developed so far also disrupt the binding of viral glycoproteins to receptor cells. Therefore, an effective method of treating viral disease is to use proteins that are capable of targeting glycans present on the glycoprotein envelop. Griffithsin is a promising broad-spectrum antiviral agent effective against numerous glycosylated enveloped viruses, e.g., EBOLA virus, HIV, SARS, etc. Though these are genetically and taxonomically different, they all are enveloped viruses and possess a lipid layer that protects the capsid and genome of the virus.

Griffithsin is widely studied and supposed to be a potent agent to inhibit HIV1 replications at the sub-nanomolar concentrations. Researchers have demonstrated its anti-HIV efficacy during *in-vivo* conditions while working on infection model SHIVSF162P3 in rhesus macaques. It binds to the surface gp120 protein and prevents HIV entry inside the cell. Griffithsin is considered the most potent inhibitor of penetration of HIV as per several studies performed to date [12]. It has also been shown to mitigate HCV infection in chimeric mice (mice with human hepatocytes which are employed as humanized models) and prevent *in vitro* HCV infection of Huh-7 hepatoma cells. There are reports of its action against the Japanese encephalitis virus (JEV) and Nipah virus also [13,14].

Table 1 summarizes the action of Griffithsin against different viruses.

Griffithsin and coronaviruses

The family Coronaviridae is characterized by enveloped viruses with positive single-stranded RNA. These viruses are further classified into two sub-families, namely Coronavirinae and the Torovirinae. The sub-family Coronavirinae is further classified into Alpha, Beta, Gama, and Deltacoronaviruses [15].

Virus	Mode of Action
HIV [2,5]	Binds to HIV envelope glycoprotein gp120 Enhances binding of antibodies specific for the CD4 binding site (CD4bs) Dendritic cells possess DC-SIGN receptors which play role in transfer of HIV-1 infection from infected dendritic cell to naïve CD4+T cells in lymph nodes. This process is inhibited by Griffithsin
HCV [2]	Interacts with the high-mannose glycans on the surface of HCV
SARS-CoV and SARS-CoV-2 [16]	Binds glycans on the surface of the SARS-CoV spike glycoprotein thus inhibiting viral entry inside the cell
JEV [13]	Interacts with the glycosylated viral proteins (Envelope, E and premembrane, prM)
HSV-2[23]	Acts by blocking HSV-2 infection by interacting with one of the four glycoproteins involved in HSV-2 entry, namely glycoprotein D
HPV (non-enveloped virus) [23]	Probably interacts with the HPV secondary receptor (alpha 6 integrin) present in basal keratinocytes, decreasing its availability on the cell surface

Table 1: Mechanism of action of Griffithsin on different viruses.

HIV: Human Immunodeficiency Virus; HCV: Hepatitis C Virus; SARS-CoV: Severe Acute Respiratory Syndrome Coronavirus; JEV: Japanese Encephalitis Virus; HSV: Herpes Simplex Virus; HPV: Human Papilloma Virus; CD4: Cluster of Differentiation 4; DC-SIGN: Dendritic Cell Intercellular Adhesion Molecule 3 (ICAM-3) Grabbing Non-integrin.

Griffithsin was reported to bind tightly at multiple sites on the spiky glycoprotein envelop of coronaviruses which enables it to act against these viruses [16]. It has been studied on several beta coronaviruses, including the current pandemic causing SARS-CoV-2.

The surface-exposed S protein on both SARS-CoV and SARS-CoV-2 are highly conserved molecules comprising of a few conserved and few unique glycan positions [17]. Infection occurs when the viral S protein attaches to ACE (angiotensin-converting enzyme inhibitor) receptors present on the host cell membrane leading to viral fusion and transfer of viral contents inside the host cell. Griffithsin acts by interfering with viral entry inside the cell. This is achieved via multivalent binding to the carbohydrate component (oligosaccharide moieties) of viral S protein present on the viral envelope. In 2010 O'Keefe., *et al*. published the *in vivo* and *in vitro* action of Griffithsin on different coronaviruses, including four strains of SARS-CoV (Urbani, Tor II, CuHK, and Frank). BALB/c mice were inoculated with mouse-adapted MA125 SARS-CoV. Mice treated with intranasal Griffithsin showed 100% survival, no weight loss along with reduced viral load and pathological changes in pulmonary tissue. The control group had a 30% survival, 25% weight loss in those who survived, along with marked pathological lung damage [16]. More evidence comes from an *in-vivo* study by Millet., *et al*. where the action of Griffithsin was studied on three cell lines (Huh-7, MRC-5, Vero-81 cells) infected with MERS-CoV (EMC/2012 strain). Griffithsin significantly reduced infectivity and production of MERS-CoV in the cell lines along with no or minimal cell damage. It mainly acts during the entry phase of the virus, i.e., the early part of the disease [18]. Similar results were found in a study conducted on porcine delta coronavirus (PD-CoV), responsible for causing diarrheal disease among piglets. Griffithsin was found to be effective at concentration 1 µg/mL or above when tested against PD-CoV infected cell lines of swine testis (ST) cells and porcine intestinal epithelial (IPI-2I) cells [19].

In-vitro inhibitory action of Griffithsin on SARS-CoV-2 was first reported by Cai., *et al*. from China in a series of experiments demonstrating its binding with the S1 subunit of spike glycoprotein. The authors also reported its synergistic action with EK1 (a newly found pan-coronavirus fusion inhibitor) and the possible role of combination therapy [20].

Route of administration and safety profile

Barton., *et al*. studied the pharmacokinetics of Griffithsin with different routes (oral, subcutaneous, and intravenous) of administration in rats. The authors reported complex elimination after parenteral administration, while the drug was not orally bioavailable [21].

The drug has been tested as topical gel due to its property to inhibit viral entry inside the human host. Griffithsin did not cause any undesirable effect when tested on a model of vaginal irritation in rabbits. The Griffithsin-based gel has been used on rhesus monkeys where gel did not lead to any pathological alterations in the mucosa of the rectum, microbiota, or the intestine's proteome [22].

Human trials are underway to test the safety and efficacy of Q-Griffithsin (a recombinant oxidation-resistant variant) nasal sprays as prophylaxis against SARS-CoV-2.

Synergistic action with other antivirals

Griffithsin has also been tested in combination with other antivirals and has shown synergistic action. Topical application of a combination of Carrageenan (another algal derivative) along with Griffithsin was found to be well effective against Human papillomavirus and Herpes simplex-2 in animal models [23]. Alsaidi., *et al*. studied the Carrageenan and Griffithsin combination against pseudo-SARS-CoV and pseudo-SARS-CoV-2 in HeLa cell lines with promising results. The authors suggest the role of the Carrageenan and Griffithsin combination as a broad-spectrum antiviral agent for respiratory viruses [24].

Feasibility of large-scale production

Griffithsin is found to be quite amenable to large-scale, cost-effective preparation. The production systems need to produce bulk quantities of high and refined quality of Griffithsin, which becomes eventually available for formulation and characterization. Griffithsin is expressed in several recombinant systems [25]. Initially, Griffithsin was expressed using E. coli systems with cultures developed in a shake flask where 12 mg of Griffithsin/liter was produced, but insoluble inclusion entities were also found in it. With the use of fermenters, more than 500 mg/liter of media was produced. This curbed the problem of bacterial endotoxins and significantly reduced the manufacturing cost. Griffithsin is also an ideal candidate for a plant-based expression system. It has also been known to be expressed in chloroplasts of *Nicotiana tabacum* and in *Nicotiana benthamiana* using Agrobacterium-mediated expression systems or recombinant tobacco mosaic virus (rTMV)-based expression systems. Studies have reported optimization of the actual purification process for bulk manufacturing of Griffithsin from laminar tissues of *N. benthamiana* using rTMV based expression vectors. This new process was able to drastically improve the recovery of Griffithsin by yielding 60–90% of the total expressed Griffithsin. Tobacco and lettuce plants are optimal hosts for Griffithsin production, where zera signal peptide is used to achieve a higher amount of recombinant protein. Besides this, other green leafy vegetables have also been found useful for their effective molecular farming. It can afford a high level of transgene expression through a selection of suitable host species, signal sequences, and strong promotors. Development of the optimized recombinant systems of Griffithsin production may bring down the labor and cost to a great extent [26,27].

The recombinant Griffithsin was assessed for its physical, chemical, thermal, and functional stability during storage conditions in preformulative studies, where it was found to be a stable molecule, degrading at high temperatures (above 65^0C). It also remained stable when exposed to light, physical agitation, freeze-thaw cycle, different ionic strength, and strong acid and basic conditions [28].

Conclusion

It can be concluded from the above discussion that Griffithsin exhibits excellent potential as a broad-spectrum antiviral agent with almost negligible *in vitro* and *in-vivo* toxicity. The antiviral activity is associated with the characteristic structural feature, forming a homodimeric complex with three carbohydrate-binding domains on each monomer.

Griffithsin is currently an upcoming natural product with good feasibility of large-scale production. This can be further exploited and investigated to be used as a prophylactic or therapeutic antiviral agent, especially against SARS-CoV-2.

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