



PURIFICATION OF THE SENDAI VIRUS HN PROTEIN

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ABSTRACT

The Sendai virus (SeV) is a single-stranded RNA virus that was isolated in 1952 which primarily infects rodents and is a laboratory study model. SeV is a member of the Paramyxoviridae family and can cause serious respiratory infections in animals. The life cycle of the virus is regulated by the envelope protein hemagglutinin-neuraminidase (HN). There are several viral proteins that play important roles in cell infection. The viral cycle begins with the attachment of the HN protein to sialic acid receptors on the cell surface, followed by fusion and entry of the virus into the cytoplasm. There, the viral RNA-dependent RNA polymerase transcribes the viral genome into mRNA, which is translated into viral proteins, resulting in the formation of virions that infect other cells. SeV is non-pathogenic for humans but can infect domestic animals and rodents. It is capable of fusing eukaryotic cells and forming syncytia, useful for the formation of monoclonal antibody hybridomas e vaccine. It has been observed that low viremia can have stimulatory effects on the immune system, while high viremia can be fatal. SeV HN induces apoptosis through the inhibition of NF-κB expression which is potentiated by tumor necrosis factor (TNF). SeV envelope proteins can be isolated by various methods, such as liquid chromatography and Triton X-100. This protein has been isolated in the laboratory without alteration of biological properties.

KEYWORDS: *Sendai virus, Paramyxoviridae family, viral protein, hemagglutinin-neuraminidase, respiratory infection*

INTRODUCTION

The Sendai virus (SeV), also known as murine parainfluenza virus type 1, is a member of the Paramyxoviridae family, genus Respirovirus (1). It is an enveloped, negative-sense, single-stranded RNA virus, which is 150-200 nm large, that was originally isolated from mice in Japan in 1952. SeV is primarily a pathogen of rodents, but it has also been found to infect other animals in laboratory settings, where it is often used as a model organism for studying viral replication and pathogenesis in mammals (2,3).

The hemagglutinin-neuraminidase (HN) protein of SeV is a crucial glycoprotein embedded in the viral envelope and plays a significant role in the virus's lifecycle (4). SeV has a typical paramyxovirus structure, featuring a helical nucleocapsid surrounded by a lipid envelope derived from the host cell membrane (5). Embedded in this envelope are viral glycoproteins critical for infection: HN, fusion (F) protein, and matrix (M) protein (4). The HN protein facilitates binding to sialic acid-containing receptors on the surface of host cells and also possesses neuraminidase activity, which helps in the release of new viral particles. The F protein mediates the fusion of the viral envelope with the host cell membrane, allowing entry of the viral genome into the host cell (6).

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The genome of SeV is approximately 15,384 nucleotides long and encodes six major proteins: nucleocapsid protein, phosphoprotein (P), M protein, F protein, HN, and the large protein, which is the viral RNA-dependent RNA polymerase (Fig.1). Additionally, there are two nonstructural proteins, C and V, produced from the P gene by alternative transcription mechanisms (7).

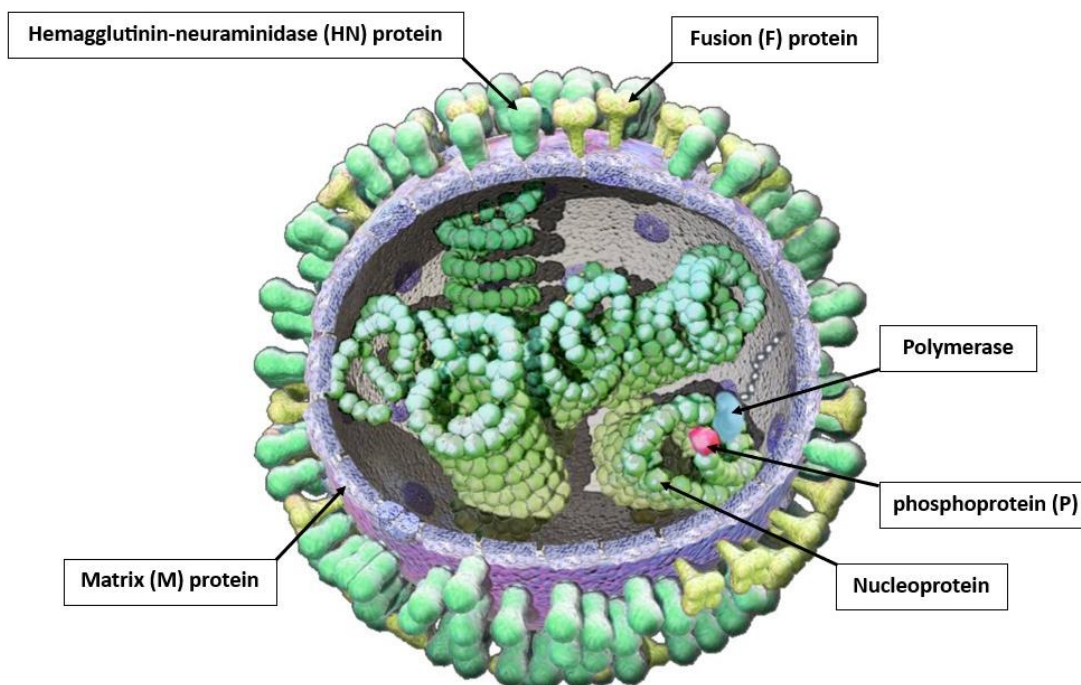


Fig. 1. Sendai Virus (SeV) causes a severe respiratory infection in mice, but only mild disease in humans. SeV has a typical paramyxovirus structure, featuring a helical nucleocapsid surrounded by a lipid envelope derived from the host cell membrane. Embedded in this envelope are the viral glycoproteins hemagglutinin-neuraminidase (HN), fusion (F) protein, and matrix (M) protein.

The SeV life cycle begins with the attachment of the HN protein to the sialic acid receptors on the host cell surface. The F protein then facilitates the fusion of the viral envelope with the host cell membrane, allowing the viral nucleocapsid to enter the cytoplasm. Once inside, the viral RNA-dependent RNA polymerase transcribes the viral genome into mRNA, which is translated into viral proteins by the host cell machinery (8).

Replication of the viral genome occurs in the cytoplasm, leading to the production of new nucleocapsids. These nucleocapsids are then transported to the cell membrane, where they are assembled into new virions with the help of the M protein. The newly formed virions bud off from the host cell, acquiring their envelope from the host cell membrane in the process, and go on to infect new cells (9).

Beyond its role as a pathogen, SeV has gained significant attention in biomedical research due to its ability to induce cell fusion (syncytium formation) and for its strong immunogenic properties (10). It has been used as a tool for studying viral replication, gene expression, and host immune responses (11). Moreover, modified versions of SeV are being explored as vectors for gene delivery and vaccine development, taking advantage of its efficient infectivity and ability to stimulate robust immune responses.

In laboratory settings, SeV is handled under biosafety level 2 (BSL-2) containment due to its potential to cause respiratory infections in rodents and, less commonly, in humans (12). Standard precautions and practices are followed to prevent accidental exposure and spread, ensuring the safety of researchers and laboratory animals.

DISCUSSION

The cell surface contains glycans that are used as receptors by viruses to enter the cell and infect tissues and organs (13). SeV is not pathogenic for humans but can infect domestic animals and rodents (14). It is also a mediator of tumor effects and is capable of fusing eukaryotic cells and forming syncytia (15).

SeV is a good microorganism for building hybridomas and monoclonal antibodies (16). SeV is also involved in the reprogramming of somatic cells into stem cells and could be useful for the generation of vaccines (17). The virus replicates in the cell cytoplasm, does not undergo genetic recombination and does not integrate into the host's genome. SeV replication takes about 14 hours and uses its own RNA polymerase that produces thousands of virions (18). Vaccine studies have been completed, and in many cases, SeV helps stimulate the formation of specific antibodies, although research is still in the preclinical phase (19).

Studies have shown that in mice, low viremia can have stimulatory effects on the immune system, while high viremia can be fatal (20). The severity of the infection may depend on the type of animal infected, but even strains of the same species may have a different susceptibility to SeV infection. Mice treated with interferon-gamma (IFN- γ) before and during viral infection have shown satisfactory resistance to SeV infection with a decreased mortality rate (21). Today, SeV envelope proteins can be isolated by various methods, such as reversed-phase high-performance liquid chromatography, non-ionic detergents decylpolyethyleneglycol-300, and Triton X-100, amongst others (22).

The surface glycoproteins of strand negative RNA viruses are surface antigens, some of which are certain to induce neutralizing antibodies (3). The isolation and purification of these glycoproteins represents an essential step for the preparation of semi-synthetic vaccines or those prepared with viral subunits (3). However, this purification is difficult for some reasons. There is the difficulty of removing the surfactants which are used both for the fragmentation of the virions and for the subsequent stages of the purification process (23). In fact, these surfactants can be harmful if inoculated into humans. Furthermore, these glycoproteins tend to form non-homogeneous aggregates of different sizes. This leads to difficulties in isolating these glycoproteins using simple gel filtration methods (22).

HN of the SeV is a membrane protein of Newcastle Disease virus (NDV) that acts on several transformed cells, sending them into apoptosis (24). The apoptotic action of the HN protein occurs through its inhibition of the expression of NF- κ B (25). In addition, pro-inflammatory protein tumor necrosis factor (TNF), which has apoptotic capabilities, increases the apoptotic power of the HN protein. It is likely that this effect is due to the sensitization of the viral HN protein on some types of cells, increasing the apoptotic power of TNF (26).

During our work, we have utilized an isolation method for the HN glycoprotein which maintains its immunogenic and biological properties (agglutinating and neuraminidase activity), without undergoing change and which is free of surfactant residues (27). This purification process uses fractionation methods designed to isolate the different viral components of RNA viruses with the pericapsid envelope and can represent a standard model for the isolation of glycoproteins with immunogenic activity from Myxovirus and Paramyxovirus (28).

After clarification of the allantoic fluid for certification at 3000 RPM for 15 minutes, SeV is concentrated and partially purified by centrifugation at 70,000 g for one hour (Spingo rotor type 3). The centrifuged product is suspended in BBS (borate buffer) pH 7.2 and purified by centrifugation in a linear sucrose gradient 10-50% weight: volume at 160,000 g in a Spinco SW 40 rotor. The viral particles are collected from the center of the gradient with an ISCO fractionator. The purified virus is dialyzed against BBS pH 7.2, concentrated until it reaches 2000 EU, and added with an equal quantity of 2% Nonidet (final concentration of 1% Nonidet), and then treated with ultrasound (3 Ampere for 3 minutes). SeV is centrifuged at 15,000 g for 60 minutes.

The non-solubilized viral protein fractions of the normal acrylamide gel electrophoretic pattern, in which the HN and F glycoproteins are strongly decreased, appears in the precipitate. These significantly purified glycoproteins are found in the supernatant. The supernatant is passed into a Lentil lectin Sepharose 4B column that is balanced to pH 5.5. Subsequently, the column is washed with 10 mM Tris, pH 5.5, to remove the surfactant and any traces of proteins not bound to the Lectin. The glycoproteins are eluted from the column with 10 mM Tris pH 6.8 containing 0.1 M mannoside and then with 10 mM Tris pH 8.3 containing 0.1 M mannoside and 1% deoxycholate. Three peaks appear in the elution graph: the first wash containing Nonidet, then the eluate at pH 6.8, and finally, the eluate at pH 8.3. The last two peaks are concentrated. Acrylamide gel electrophoresis reveals a small eluate at pH 6.8 and the presence of a high concentration of NH together with traces of F and F', which are connected by disulphide bonds. In the fraction eluted at pH 8.3, there are evident traces of the HN fraction and most of the F and F' fractions. The fraction eluted at pH 6.8 in which the majority of the immunogenically active HN glycoprotein is present, as detected by immunodiffusion tests, and which maintains the neuraminidase activity (Warner reaction) and the haemagglutinating activity, is passed into an Aca 44 column to further purify it from traces of glycoprotein F through gel filtration.

CONCLUSIONS

SeV, with its well-characterized life cycle and genetic makeup, continues to be a valuable model in virology and an important tool in biotechnological applications. Investigations into the mechanisms of infection and replication not only provide insight into viral pathogenesis but also open avenues for innovative therapeutic and preventive strategies. The purification model that we utilized is useful for understanding the features and physiopathology of the virus.

Conflict of interest

The authors declare that they have no conflict of interest.

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