Expression of the Sendai virus fusion protein in insect cells and characterization of its post-translational modifications

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The fusion (F) protein of Sendai virus was expressed with a baculovirus system in insect cells. The F protein is synthesized in its uncleaved form F0, which appears in SDS-containing gels as two bands with Mr values of 66K and 64K. Digestion of the F protein with endoglycosidase H revealed that the 66K species contains high mannose-type carbohydrates, whereas partially processed oligosaccharides are attached to the 64K species. Pulse-chase experiments showed that the F protein is initially synthesized as its 66K form. After 1 h of chase this precursor glycoprotein is partially converted to the 64K species and exposed at the cell surface. Other modifications of the F protein in insect cells are the formation of intramolecular disulphide linkages and oligomerization. However, processing and plasma membrane transport of the F protein in insect cells are incomplete and retarded compared to the F protein synthesized in Sendai virus-infected mammalian cells.

Sendai virus, a member of the Paramyxovirus family, is a negative-strand RNA virus. Like other paramyxoviruses, it consists of a helical nucleocapsid surrounded by a glycoprotein envelope. The viral envelope is composed of a lipid bilayer, derived from the plasma membrane of the host cell, and two virus-encoded glycoproteins: the fusion (F) protein and the haemagglutinin-neuraminidase (HN) protein. The HN protein possesses cell binding and neuraminidase activities, whereas the F protein is essential for virus penetration into host cells (Galinski & Wechsler, 1991).

In infected vertebrate host cells the F protein is synthesized on membrane-bound ribosomes as an inactive precursor (F0) with an Mr of 67K. The fusion property of this precursor glycoprotein is activated by proteolytic cleavage at a single arginine residue by trypsin-like endoproteases to form two disulphide-linked subunits, F1 (51K) and F2 (16K) (Ohuchi & Homma, 1975; Scheid & Choppin, 1977). Other post-translational modifications of the F protein in vertebrate cells include the cleavage of the signal peptide, the formation of non-covalently linked tetramers and the addition, processing and terminal glycosylation of N-linked carbohydrates (Yoshima et al., 1981; Sechoy et al., 1987; Morrison, 1988). Attachment of fatty acids, which has been described for other paramyxovirus F proteins, was not observed for the F protein of Sendai virus (Veit et al., 1989).

The nucleotide sequences of the F proteins from a number of paramyxoviruses, including two Sendai virus strains, have been determined (Blumberg et al., 1985a; Shioda et al., 1986). All F proteins show homology in their amino acid sequences with conservation of cysteine residues, and reveal the features typical of a type I membrane glycoprotein with a signal sequence at the N terminus of the F1 subunit and a transmembrane region in the F2 subunit. A third hydrophobic domain is present at the N terminus of the F2 subunit, which is involved in the fusion process (Morrison, 1988).

Cloned F genes of different paramyxoviruses have been expressed in insect and mammalian cells, which has yielded valuable information about the structure and function of these proteins (Paterson et al., 1985; Wathen et al., 1989; Alkhatib et al., 1990; Vialard et al., 1990). However, expression of the F protein of Sendai virus has been reported only recently for mammalian cells, but the gene products and their post-translational modifications were not characterized further (Sakaguchi et al., 1993; Sanderson et al., 1993).

In this report, we describe the expression of the F glycoprotein of Sendai virus in insect cells using a baculovirus system based on Autographa californica nuclear polyhedrosis virus (AcNPV).

A full-length F gene was cloned into the expression plasmid pVL1393 behind the strong promoter of the baculovirus polyhedrin gene. This construct together with Bacpak6 AcNPV genomic DNA (Kitts & Possec, 1993) were cotransfected into Spodoptera frugiperda cells (Sf-21) and a recombinant virus stock containing the F gene (AcNPV–F) was prepared as described by King &
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(a) Expression of the F protein in insect cells. Sf-21 cells were infected with AcNPV-F (lanes 1 and 2), wild-type baculovirus (lane 4) or were mock-infected (lane 3). Two days p.i. the cells were labelled for 4 h with [35S]methionine (50 μCi/ml). Cell extracts were immuno-precipitated with a polyclonal Sendai virus antiserum (lanes 2, 3 and 4) or a MAb against the F protein (lane 1) and subjected to SDS-PAGE on a 10 % acrylamide gel followed by fluorography. The electrophoretic mobility of M_r markers is indicated on the left. (b) Electrophoretic mobility of the F protein from insect and mammalian cells. The F protein was immunoprecipitated from AcNPV-F-infected Sf-21 cells with the polyclonal antiserum (lanes 1 and 3) or from Sendai virus-infected CV-1 cells with the MAb (lanes 2 and 4) and analysed by SDS-PAGE using reducing (lanes 3 and 4) or non-reducing conditions (lanes 1 and 2). The resulting fluorogram is shown.

Possee (1992). Sf-21 cells were infected either with this virus stock, with non-recombinant baculovirus or were mock-infected. Cells were labelled for 4 h with [35S]methionine and cell extracts were immunoprecipitated with both a monoclonal antibody (MAb) against the F protein and a polyclonal Sendai virus antiserum. After SDS–PAGE and fluorography, two protein bands were observed in recombinant baculovirus-infected cells, but not in control cells, which provides evidence that both polypeptides are products of the F gene (Fig. 1a).

To characterize the F gene products further, the F protein obtained from insect cells was co-electrophoresed with the F protein immunoprecipitated from Sendai virus-infected mammalian cells (CV-1) using reducing as well as non-reducing conditions (Fig. 1b). In the presence of mercaptoethanol (lanes 3 and 4) the upper band of the F protein from insect cells has nearly the same M_r (66K) as that immunoprecipitated from CV-1 cells (67K). However, the lower F protein band (64K) from insect cells has no counterpart in mammalian cells. A small amount of the cleaved F1 subunit (weak band at 51K in lane 4) is detectable in mammalian but not in insect cells. This indicates that a trypsin-like endoprotease with a substrate specificity for a single arginine residue (Ogasawara et al., 1992; Tashiro et al., 1992) is not present or not active in Sf-21 cells.

In the absence of mercaptoethanol, the F protein from mammalian cells has a higher electrophoretic mobility (64K), probably due to the more compact folding of the glycoprotein with intact disulphide linkages. The same shift in the electrophoretic mobility under non-reducing conditions was also observed for both F protein species synthesized in insect cells (Fig. 1b, lanes 1 and 2), which suggests that these proteins can form intramolecular disulphide linkages.

To explain the two F protein species of 64K and 66K in insect cells, we suspected differences in their glycosylation. Therefore we treated immunoprecipitated F protein with glycopeptidase F (PNGase-F), an enzyme that removes all types of N-linked carbohydrates. SDS–PAGE and fluorography revealed a major protein band with an M_r of 60K (Fig. 2a), as would be expected for a polypeptide chain without any modifications. This suggests that the two protein bands of the F protein in Sf-21 cells are indeed caused by different types or amounts of N-linked carbohydrates. A weak band with an M_r slightly above 60K was also present in PNGase-F-treated samples. This may be due to incomplete deglycosylation.

To investigate glycosylation in more detail, the immunoprecipitated F protein was also treated with endoglycosidase H (endo-H), which removes only carbohydrates of the high mannose type. The results in Fig. 2(a, lane 2) show that the major product of the F gene (64K) is not shifted and hence contains endo-H-resistant, processed carbohydrates, whereas the 66K species shifts to the same position as the PNGase-F-treated protein and therefore contains unprocessed carbohydrates of the high mannose type. The observation that the F protein with processed carbohydrates has a lower M_r (64K) than the natural F protein synthesized in mammalian cells (67K), suggests that glucose and some mannose residues have been removed from the high mannose precursor, but terminal glycosylation has not occurred. The presence of truncated oligosaccharides is in accordance with reports on other viral glycoproteins expressed in insect cells (Kuroda et al., 1990; Vialard et al., 1990; Fraser, 1992).

It has also been demonstrated that terminal glycosyl-
immmunoprecipitated with the polyclonal antiserum and subjected to SDS-PAGE and fluorography. The F protein was infected with AcNPV-F and labelled with $[^{35}\text{S}]$methionine at 1, 2, 3 and 4 days p.i., as indicated above the lanes. The F protein was immunoprecipitated with the polyclonal antiserum and subjected to SDS-PAGE and fluorography.

Fig. 2. Glycosylation of the F protein in insect cells. (a) F protein labelled for 4 h with $[^{35}\text{S}]$methionine was digested after immunoprecipitation with endo-H (lane 2), PNGase-F (lane 3) or left untreated (lane 1) prior to SDS-PAGE and fluorography. (b) SF-21 cells were infected with AcNPV-F and labelled with $[^{35}\text{S}]$methionine at 1, 2, 3 and 4 days p.i., as indicated above the lanes. The F protein was immunoprecipitated with the polyclonal antiserum and subjected to SDS-PAGE and fluorography.

Fig. 3. Kinetics of F protein processing in insect cells. SF-21 cells infected with recombinant AcNPV-F were labelled with $[^{35}\text{S}]$methionine for 15 min and chased for 0, 30, 60 or 120 min (lanes 1, 2, 3 and 4, respectively). Ten minutes prior to cell lysis, trypsin was added to the incubation medium (10 µg/ml). After immunoprecipitation with the polyclonal antiserum, samples were subjected to SDS-PAGE followed by fluorography. The small subunit $F_0$ is not visible in 10% acrylamide gels.

ation of several recombinant glycoproteins, which causes an upward shift in the electrophoretic mobility, is induced late during baculovirus infection (Davidson & Castellino, 1991). However, we did not observe any differences in the $M_r$ values of the F protein species synthesized 1, 2, 3 or 4 days after baculovirus infection of insect cells (Fig. 2b). The result of the time course experiment revealed further that synthesis of the F protein can be detected as early as 24 h post-infection (p.i.). As is apparent in Fig. 2(b) the largest amount of F protein was synthesized at 48 h p.i. after which the expression rate decreased again.

To study a possible precursor–product relationship of the two F protein forms and the kinetics of transport of the F protein to the plasma membrane, infected SF-21 cells were pulse-chase-labelled with $[^{35}\text{S}]$methionine. Ten minutes before cell lysis, intact cells were incubated with trypsin, which cleaves any $F_0$ precursor appearing on the cell surface into $F_1$ and $F_2$ subunits. The F protein was then immunoprecipitated from the cell lysates and subjected to SDS-PAGE and fluorography (Fig. 3). Only the 66K species of F protein with high mannose-type oligosaccharides was detectable during the pulse and up to 30 min of chase (lanes 1 and 2), which provides evidence that this is the precursor for the 64K F protein species expressed in insect cells. The 64K F$_0$ species with processed carbohydrates as well as the trypsin-cleaved and surface-exposed F$_1$ subunit are first detectable after 1 h of chase. The 64K population of $F_0$ occurs also in the absence of trypsin and is most prominently seen after longer labelling times (Fig. 1, 2 and 4). Processing of carbohydrates and surface exposure of the F protein are not completed after 2 h of chase (see lane 4), which suggests that both processes are retarded and inefficient in insect cells when compared to mammalian cells, in which half-times for surface expression of the F protein of 15 to 20 min have been reported (Blumberg et al., 1985b). In our hands the F protein in Sendai virus-infected CV-1 cells is completely cleaved into $F_1$ after 2 h of chase (not shown in figures).

This may be partially due to different cell culture conditions for mammalian and insect cells, e.g. different temperatures (37 °C rather than 27 °C) and pH values of the media (7.4 compared to 6.05). However, one rate-limiting step for transport and processing of glycoproteins is their correct oligomerization in the endoplasmic reticulum (Doms et al., 1993). It was therefore of interest to analyse in insect cells the oligomerization of the F protein, which is present in Sendai virus particles as a tetramer composed of two dimers (Sechoy et al., 1987). This was done after lysing cells with non-denaturing NP40 buffer by chemical cross-linking with 3,3′-dithiobis(propionic acid N-hydroxysuccinimide ester) (DSP). As shown in Fig. 4(a) (lane 2), PAGE analysis of the cross-linked samples revealed bands with apparent $M_r$ values of 123K and about 250K, which is consistent with the calculated $M_r$ of F protein dimers and tetramers. When analysed without DSP, only monomers were observed. However, a large amount of monomer remains after cross-linking (Fig. 4a, lane 2), which suggests either that oligomerization in insect cells is incomplete or that our experimental conditions are insufficient for a complete cross-linking of oligomers. To distinguish between these possibilities, a similar experiment was done with the F protein synthesized in mammalian cells (Fig. 4b). In this case, complete cross-linking of the F protein in tetramers was observed, which provides evidence that oligomerization of F protein in insect cells is indeed incomplete. This is in accordance with previous studies on the processing of influenza virus
Fig. 4. Oligomerization of the F protein in insect and mammalian cells. SF-21 cells (a) and CV-1 cells (b) synthesizing the F protein were labelled with [35S]methionine for 4 h and cells were lysed with non-denaturing buffer (0.5% NP40, 150 mM-NaCl, 5 mM-EDTA, 50 mM-Tris-HCl pH 7.9). Cleared lysates (200 μl) were incubated with DSP for 15 min at 15 °C. Cross-linking was terminated by the addition of 4 μl of 1 M-NH₄HCO₃. The F protein was immunoprecipitated with the polyclonal antiserum (a) or the MAb (b) and subjected to SDS-PAGE in 7% gels under non-reducing conditions and fluorography. M, Monomer; D, dimer; T, tetramer.

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References


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