Single Amino Acid Substitution of Sendai Virus at the Cleavage Site of the Fusion Protein Confers Trypsin Resistance

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SUMMARY

Amino acid sequences of fusion (F) proteins of two trypsin-resistant mutants of Sendai virus, TR-2 and TR-5, were deduced from nucleotide analysis of cDNA encoding the F gene and were compared with that of the trypsin-sensitive wild-type Sendai virus. In both mutants, amino acid substitutions were found at residues 116 (Arg → Ile), the cleavage site of the F protein, and 109 (Asn → Asp). Two trypsin-sensitive revertants, TSrev-52 and TSrev-58, derived from TR-5 were both activated by trypsin similarly to the wild-type virus and had a single amino acid reversion from Ile to Arg at residue 116, leaving Asp as before at residue 109. These results indicate that the trypsin sensitivity of Sendai virus can be changed by a single amino acid substitution at the cleavage site of the F protein and a mutation from Arg to Ile is responsible for the acquisition of resistance to trypsin.

Sendai virus (HVJ), has two envelope glycoproteins (Mountcastle et al., 1971) designated HANA and F (Homma et al., 1975). HANA protein possesses both haemagglutinating and neuraminidase activities through which the virus adsorbs to the host cell receptors (Tozawa et al., 1973; Scheid & Choppin, 1974). F protein is a fusion protein and mediates the second step of infection by fusing the viral envelope and the plasma membrane, releasing the nucleocapsid into host cells (Homma & Ohuchi, 1973; Scheid & Choppin, 1974). F protein is initially synthesized as a precursor glycoprotein of Mr 65,000; virus having this form of F protein is inactive and exhibits no haemolytic and cell-fusing activities or infectivity. Conversion of virus from the inactive to the active form can be achieved by a mild trypsin treatment in vitro which is accompanied by cleavage of the precursor F protein into its F₁ (Mr, 51,000) and F₂ (Mr, 15,000) subunits (Homma, 1971, 1972a; Homma & Ohuchi, 1973; Homma & Tamagawa, 1973; Scheid & Choppin, 1974; Ohuchi & Homma, 1976). Since the cleavage of the F glycoprotein occurs post-translationally and is accomplished by some protease(s) in the chorioallantoic fluid of embryonated chicken eggs (Muramatsu & Homma, 1980), in tissue culture cells (Shibuta et al., 1971; Silver et al., 1978) or in mouse lung (Tashiro & Homma, 1983a), we and other authors have suggested that the presence of the activating enzyme(s) for Sendai virus will determine its host range and organ tropism (Homma, 1972b; Ishida & Homma, 1978; Silver et al., 1978). A similar proposal has been made concerning the virulence of Newcastle disease virus (Garten et al., 1980; Nagai et al., 1979).

In previous reports we isolated a protease activation mutant designated TR-2, by passaging wild-type Sendai virus in the presence of chymotrypsin. TR-2 can be activated in vitro by chymotrypsin but not by trypsin. TR-2 previously activated by chymotrypsin in vitro underwent only one step of replication in mouse lung where no activating proteases for TR-2 were found and brought about negligible lung lesions (Tashiro & Homma, 1983b, 1985). Scheid & Choppin (1976) isolated some protease activation mutants and the sites of amino acid substitutions in
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Fig. 1. Virus growth of the trypsin-sensitive revertants in the presence of various concentrations of trypsin. LLC-MK2 cells were infected with 0.01 p.f.u./cell of virus in the presence of various concentrations of trypsin at 37 °C for 120 h. The progeny viruses released from the cells were measured for haemagglutinating activity as haemagglutinating units/ml (HAU/ml). ○, Wild-type; □, TR-5; ▲, TSrev-52; ■, TSrev-58.

these mutants have been determined very recently (Hsu et al., 1987), although the analyses were limited to only 60 to 70 amino acids surrounding the activation cleavage site. There is evidence that a single amino acid substitution occurring at a residue distant from the cleavage site of the F protein could cause conformational change around the NH2 terminus of the F1 subunit (Portner et al., 1987). Accordingly, it is possible that such a mutation may contribute to the alteration of the protease sensitivity of the F protein. Working independently of the above authors, we have determined the full nucleotide sequences of cDNA representing the F genes of the trypsin-sensitive wild-type Sendai virus, its trypsin-resistant mutants and trypsin-sensitive revertants. By comparison with the whole amino acid sequences deduced from their nucleotide sequences, we aimed to find the mutation point(s) of TR-2 responsible for the trypsin resistance.

Three types of viruses were used: the trypsin-sensitive wild-type Fushimi strain of Sendai virus, trypsin-resistant mutants of the Fushimi strain TR-2 (Tashiro & Homma, 1983b) and TR-5 (a further isolate from plaques of TR-2) and the trypsin-sensitive revertants TSrev-52 and TSrev-58 which were obtained by passaging TR-5 in the presence of 3 μg/ml trypsin. TR-5 was chosen as the parent virus for isolation of revertants because it had a marker amino acid Met at residue 172 (Fig. 4) and the resulting trypsin-sensitive revertants, TSrev-52 and TSrev-58, could be distinguished from the wild-type virus.

As shown in Fig. 1, TSrev-52 and TSrev-58 regained trypsin sensitivity and underwent multiple cycles of replication in the presence of trypsin, similar to the wild-type virus, in contrast to their parent virus, TR-5. When analysed by polyacrylamide gel electrophoresis, the F protein of the revertants was hydrolysed into the F1 and F2 subunits after treatment with trypsin (data not shown), indicating that activation of the revertants by trypsin is related to the cleavability of F by trypsin and that some mutation(s) must have taken place in the gene coding for the F protein, converting TR into the trypsin-sensitive revertant.

To elucidate the trypsin resistance of TR mutants, we first determined the nucleotide sequences of the F gene of the wild-type virus, the TR mutants and the TS revertants, and then compared the amino acid sequences deduced therefrom. Cloning of cDNA was performed according to the method of Okayama & Berg (1982) and the clones containing sequences corresponding to the F gene were identified by hybridization with 35S-labelled cDNA of the F protein gene of the Sendai virus Z strain. Sequence analysis of the nucleotides was carried out by the dideoxy chain-termination method of Sanger et al. (1977).

Fig. 2 shows the nucleotide sequence of the F protein gene of the wild-type Fushimi strain which is the original strain of Sendai virus, and the amino acid sequence deduced therefrom.
Fig. 2. Nucleotide sequence of mRNA-sense (+) DNA of the F gene of the wild-type Sendai virus Fushimi strain and its predicted amino acid sequence. In the amino acid sequence, the cleavage/activation site by trypsin is shown by an arrow above the sequence and the putative N-linked carbohydrate attachment sites are underlined.
There is a single long open reading frame encoding 565 amino acids. When the predicted amino acid sequence was compared with those of other strains of Sendai virus previously reported (Hsu & Choppin, 1984; Blumberg et al., 1985; Shioda et al., 1986), minor changes were detected (Fig. 3). The Fushimi strain contained three functionally important hydrophobic sequences corresponding to the transmembrane (anchor) region near the C terminus, the N terminus region of the F1 subunit and the signal peptide at the N-terminal end of the F2 subunit, respectively. The amino acid sequences of these regions were the same for all strains except one amino acid at residue 519 in the anchor region, where Val was replaced by Ile in the Z strain. The Fushimi strain had three putative N-linked carbohydrate attachment sites as reported by Blumberg et al. (1985) but the Z strain possessed a fourth site. The location of cysteines, which are important for the three-dimensional structure of the protein, was the same in the Fushimi and Z strains. Thus, the fundamental structure of the F protein of the Fushimi strain is well preserved.

In the case of TR-2, two nucleotide changes were noted, at positions 378 (A → G) and 400 (G → T), both resulting in amino acid substitutions, at residues 109 (Asn → Asp) and 116 (Arg → Ile), respectively. With TR-5, there was an additional nucleotide change at position 567 (G → A) corresponding to an amino acid substitution (Val → Met) at residue 172 (Fig. 4). Since TR-5 was resistant to trypsin and in this respect is indistinguishable from TR-2, this change may not be responsible for the trypsin resistance.

To decide the amino acid change responsible for the trypsin resistance of the TR mutants, the nucleotide sequences of two trypsin-sensitive revertants were examined. We found a transversion from T to G at position 400 in both revertants, but nucleotides G and A at positions 378 and 567, respectively, were conserved (Fig. 4). The former change may cause the reversion of Ile at residue 116 back to Arg. Among the amino acid changes, the Arg → Ile mutation is the one most likely to induce the trypsin resistance of the TR mutants because trypsin cleaves the F protein at this point (Gething et al., 1978; Richardson et al., 1980). However, the possible involvement of the Asn → Asp mutation at residue 109 should be considered. This is situated near the cleavage site and the simultaneous change of Asn → Asp and Arg → Ile might be necessary to cause trypsin resistance. The present results with the revertants exclude this possibility since Asp at residue 109 remained unchanged irrespective of their trypsin sensitivity. The results show that a single amino acid change at residue 116 is responsible for the change in the trypsin sensitivity of the F protein; when the amino acid is Arg, the F protein is sensitive to
trypsin, whereas if it is Ile, it is resistant. In contrast to the TR mutants, the revertants regained pathogenicity for the lungs of mice (unpublished data), suggesting that a single amino acid mutation is sufficient to alter the pneumopathogenicity of Sendai virus in mice.

Our results obtained with TR mutants are compatible with the results reported by Hsu et al. (1987) concerning their protease activation mutant, pa-c1, which lost trypsin sensitivity and simultaneously acquired chymotrypsin sensitivity. It should be noted that both the TR and pa-c1 mutants, although isolated independently, have the same mutations at residues 116 (Arg → Ile) and 109 (Asn → Asp). Since the mutation of Arg to Ile has been shown to be responsible for trypsin resistance, the other mutation may be involved in chymotrypsin sensitivity. A difference exists, however, in the chymotrypsin sensitivity of the wild-type Fushimi strain and the parental strain of pa-c1; the former could be activated by chymotrypsin (Tashiro & Homma, 1983b, 1985) whereas the latter could not (Scied & Choppin, 1976; Hsu et al., 1987). Since the complete amino acid sequence of the F protein of the latter virus is not available the above difference cannot be elucidated at present. Whether the increased chymotrypsin sensitivity of the TR mutants relates to the mutation of Asn to Asp at residue 109 has yet to be studied; experiments to resolve this question are now under way in our laboratory.

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REFERENCES


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