Single Amino Acid Change at the Cleavage Site of the Fusion Protein Is Responsible for Both Enhanced Chymotrypsin Sensitivity and Trypsin Resistance of a Sendai Virus Mutant, TR-5

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SUMMARY

A trypsin-resistant mutant of Sendai virus, TR-5, which was obtained by passaging the wild-type (wt) virus in the presence of chymotrypsin followed by plaque purification, also had an enhanced susceptibility to activation by chymotrypsin. A trypsin-sensitive revertant, TSrev-58, derived from TR-5 had a decreased susceptibility to chymotrypsin and was susceptible to trypsin to the same degree as the wt virus. Based on the facts that TR-5 had an amino acid change (Arg→Ile) at residue 116 of the fusion (F) protein, which is the cleavage site for trypsin in the wt virus F protein, and that this change had reverted in TSrev-58, it was concluded that the above amino acid change was responsible for both the enhanced chymotrypsin sensitivity and the trypsin resistance of TR-5. In this paper, the cleavage site of the F protein of TR-5 by chymotrypsin was determined in comparison with those of the wt virus and TSrev-58. The cleavage sites were not different among these viruses, being located between residues 114 (Gln) and 115 (Ser), the two residues at the N-terminal side of the trypsin cleavage site. A possible reason for the increased susceptibility of TR-5 to chymotrypsin is discussed.

Sendai virus (HVJ) has two envelope glycoproteins (Mountcastle et al., 1971) designated HANA and F (Homma et al., 1975). Sendai virus penetrates the host cell through adsorption of the HANA protein to the cellular receptors (Tozawa et al., 1973; Scheid & Choppin, 1974) and subsequent fusion of the viral envelope with the plasma membrane which is mediated by the F protein (Homma & Ohuchi, 1973; Scheid & Choppin, 1974). The envelope fusion activity of the F protein is acquired post-translationally by proteolytic cleavage of an inactive precursor F molecule (M₄ 65000) into the F₁ (M₄ 51000) and F₂ (M₄ 15000) subunits (Homma & Ohuchi, 1973; Scheid & Choppin, 1974; Ohuchi & Homma, 1976), and by this process a hydrophobic amino acid sequence appears at the N terminus of the F₁ subunit (Scheid & Choppin, 1977). Similar proteolytic activation has been observed among paramyxoviruses other than Sendai virus, such as human parainfluenza virus type 1 (Nakamura & Homma, 1974) and type 3 (Spriggs et al., 1986), bovine parainfluenza virus type 3 (Shibuta et al., 1986), Newcastle disease virus (Nagai et al., 1976), simian virus 5 (Peluso et al., 1977), measles virus (Graves et al., 1978) and respiratory syncytial virus (Lambert & Pons, 1983). The hydrophobic amino acid sequence at the N terminus of the F₁ subunit is highly conserved among these viruses (Spriggs et al., 1986; Suzu et al., 1987; McGinnes & Morrison, 1986; Paterson et al., 1984; Richardson et al., 1986; Collins et al., 1984) and its interaction with the cytoplasmic membrane is necessary for triggering envelope fusion (Gething et al., 1978). Since activation of Sendai virus was shown to be accomplished by a host protease(s) both in ovo (Muramatsu & Homma, 1980) and in the lungs of mice (Tashiro & Homma, 1983a), the presence of such an enzyme(s) was thought to determine the host range and organ tropism of Sendai virus (Homma, 1972; Ishida & Homma, 1978; Tashiro & Homma, 1983b).
Protease activation, i.e. trypsin-resistant, mutants, TR-2 and TR-5, which were both activated by chymotrypsin but not by trypsin in vitro, caused only negligible lesions in the lungs of mice from which the activating protease for the mutants was absent (Tashiro & Homma, 1983b, 1985). Recently, we found two amino acid substitutions in the F protein of both of the TR mutants compared to the wild-type (wt) virus, at residues 109 (Asn→Asp) and 116 (Arg→Ile). Trypsin-sensitive revertants, TSrev-52 and TSrev-58 derived from TR-5, became activated by trypsin in the same manner as the wt virus and had restored pneumopathogenicity in mice (Mochizuki et al., 1988). Since both revertants had a single amino acid reversion from Ile to Arg at residue 116, leaving the Asp at residue 109 unchanged, we concluded that the change at residue 116 resulted in the trypsin resistance of the TR mutants (Itoh et al., 1987).

We previously showed that the wt virus itself had a certain degree of susceptibility to chymotrypsin which was increased by the mutations in TR-2 (Tashiro & Homma, 1985). In this paper, studies were done to find out which one of the two amino acid substitutions at residues 109 and 116 was involved in the increased chymotrypsin susceptibility of the TR mutant. We noticed in preliminary studies that both of the TSrevs had become less sensitive to chymotrypsin compared to the parental TR-5 mutant. Accordingly, to resolve the above problem the chymotrypsin susceptibility of TSrev-58 was first examined in detail and the result was compared with those obtained for the wt virus and TR-5. We then determined the chymotrypsin cleavage site of the F protein of these viruses to see whether TR-5 had a cleavage site which differed from the others in such a way as to cause enhanced chymotrypsin susceptibility.

The viruses used were the wt Fushimi strain of Sendai virus, the mutant TR-5 and TSrev-58, which are mentioned above and also described in detail in a previous paper (Itoh et al., 1987). The activation experiments were performed using the methods described previously (Tashiro & Homma, 1985) with the following modifications. Unactivated viruses grown in LLC-MK2 cells were treated with various concentrations of proteases in the presence of 0.2% bovine serum albumin and the incubation was longer than the original, for 14 h at 37 °C. This modification was suitable for detecting slight differences in chymotrypsin sensitivity between TR-5 and the wt virus or TSrev-58. For determination of the chymotrypsin cleavage site, the envelope proteins of each of the purified viruses were separated from the core proteins by centrifugation after solubilization with Triton X-100 (Scheid & Choppin, 1973). They were then subjected to SDS-PAGE and the F1 proteins were extracted from the gel. The amino acids were analysed sequentially from the N-terminal end of F1, using an amino acid sequencer (Applied Biosystems 470A) and a PTH-amino acid analyser (Applied Biosystems 120A).

As shown in Fig. 1(a), TSrev-58 was activated by trypsin in exactly the same manner as the wt virus, while TR-5 was unaffected. In contrast, TR-5 was almost fully activated by the treatment with chymotrypsin at 0.25 μg/ml (Fig. 1b). Both TSrev-58 and the wt virus, however, remained inactive after the treatment with chymotrypsin, even at a concentration of 0.5 μg/ml. They became activated by chymotrypsin at more than 1 μg/ml, although the maximum infectivity titre was less than 10% of that obtained with TR-5.

In our previous report, the complete amino acid sequences of the F proteins of the three viruses used in this experiment were deduced from nucleotide analyses of their cDNA (Itoh et al., 1987), a core part of which is shown in Fig. 2. By comparing these sequences, a single amino acid change (Arg→Ile) in TR-5 at residue 116, the trypsin cleavage site of the F protein, was shown to be the cause of this mutant's resistance to trypsin (Itoh et al., 1987). As shown in Fig. 1, no difference was found between the wt virus and TSrev-58 in their sensitivity to chymotrypsin and trypsin, even though TSrev-58 had one amino acid change (Asn→Asp) at residue 109 compared to the wt virus (see Fig. 2), indicating that this amino acid change is involved in neither the chymotrypsin nor the trypsin sensitivity. In addition, the fact that TSrev-58 had only one amino acid reversion (Ile→Arg) at residue 116 compared to its parent virus TR-5 shows that this change is responsible for the changes in both the chymotrypsin and the trypsin sensitivity of TSrev-58.

In the next experiment, we attempted to find out whether the cleavage site of the TR-5 F protein is different from those of the wt virus and TSrev-58. The cleavage sites were determined by the methods described above. With all three viruses the same cleavage sites were found, between Gln and Ser at residues 114 and 115 (Fig. 2).
The results obtained in the present study overall showed that a single amino acid mutation from Arg to Ile at residue 116 in the F protein of Sendai virus is responsible for both the increase in sensitivity to chymotrypsin and resistance to trypsin. With regard to the mechanism by which the chymotrypsin sensitivity of TR-5 is enhanced, there may be two possibilities. One is an increase in the cleavability of the TR-5 F protein by chymotrypsin and the other is an enhancement of envelope fusion activity mediated by the hydrophobic structure exposed at the N terminus of the F1 subunit after cleavage by chymotrypsin. Both of these alternatives may occur. The single amino acid change from Arg to Ile may cause a conformational change of the F

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protein near the cleavage site, which may influence its cleavability by chymotrypsin and/or the envelope fusion activity. The result shown in Fig. 1, that TR-5 became activated by chymotrypsin at lower concentrations than the wt virus and TSrev-58, could be a reflection of the enhanced cleavability of TR-5 by chymotrypsin. On the other hand, the fact that the maximum infectivity titre obtained with TR-5 after treatment with chymotrypsin was higher than those obtained with the wt virus and TSrev-58 could be due to a difference in the N-terminal structure of the F subunits of these viruses.

The results obtained with TR-5 were comparable with those obtained with pa-c1 described by Scheid & Choppin (1976) in both the chymotrypsin susceptibility and the trypsin resistance. In addition, both viruses had the same mutations at residues 109 (Asn→Asp) and 116 (Arg→Ile) (Hsu et al., 1987). A marked difference was found, however, in that the parent virus of pa-c1 was reported to be resistant to chymotrypsin. Since the analysis of the sequence of pa-c1 was limited to some 60 amino acids near the cleavage site and the complete amino acid sequence of the F protein is not yet available, a direct comparison of these viruses cannot be made at present. Nevertheless, it may be worth noting that both TR-5 and pa-c1, isolated independently in different laboratories by passing the virus in the presence of chymotrypsin, had the same mutations at residues 109 (Asn→Asp) and 116 (Arg→Ile) and acquired resistance to trypsin and susceptibility to chymotrypsin. From these results, it may be concluded that either Asp is indispensable or Asn should be dispensable for enhanced susceptibility to chymotrypsin. The above view, although possible, is not sufficient because TSrev-58, having Asp instead of Asn at residue 109, was shown to be less sensitive to chymotrypsin than TR-5 (Fig. 1).

A question has arisen as to why and how the TR mutants acquired trypsin resistance simultaneously with enhanced susceptibility to chymotrypsin during the passage of the wt virus in the presence of chymotrypsin. This has now become clear in the present study with TR-5 in which a single amino acid change at residue 116 from Arg to Ile was responsible for both the enhanced susceptibility to chymotrypsin and the resistance to trypsin.

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REFERENCES


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