Restoration of the Fusion Activity of L Cell-borne Sendai Virus by Trypsin

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A close relationship between haemolytic and fusion activities of Sendai virus has been argued (Hosaka, 1962, 1970; Okada, 1958). A decrease in fusion activity of Sendai virus and Newcastle disease virus (NDV) after single passage in tissue culture cells has also been reported (Hosaka, 1962; Young & Ash, 1970).

Sendai virus (L Sendai) recovered from L cells after single passage of Sendai virus previously grown in eggs (egg Sendai) had a higher density and was apparently lacking in both infectivity for L cells and haemolytic activity (Ishida & Homma, 1960, 1961). These defective properties of L Sendai were shown to be due to the masking of sites, as a mild treatment of L Sendai with trypsin markedly restored both activities (Homma, 1971, 1972).

We have now made a comparative study of the effect of trypsin on the restoration of the fusion and haemolytic activities and on the infectivity for L cells.

L Sendai recovered from L cells after single passage of egg Sendai was prepared as described (Homma, 1971). L Sendai was treated with various concentrations of trypsin exactly following the method as used for the restoration of the infectivity for L cells and the haemolytic activity (Homma, 1971, 1972). In short, 0·3 ml of partially purified L Sendai in phosphate-buffered saline, pH 7·2, received 0·1 ml of crystalline trypsin (thrice crystallized; C. F. Bohringer and Soehne GmbH, Manheim, Germany) and incubated for 6 min at 36 °C. The enzymic action was immediately stopped by addition of 0·1 ml of crystalline soybean trypsin inhibitor (Sigma Chemical Co., St Louis, Mo.). The fusion activity was measured on two cell systems, Vero cells in monolayer and Ehrlich's ascites tumour cells in suspension. Vero cells were grown in Eagle's minimum essential medium (MEM) with 10 % calf serum. Ehrlich's ascites tumour cells were harvested from adult mice 3 days after implantation, washed and resuspended by MEM with 2 % calf serum. The procedure for fusion reaction of Ehrlich's tumour cells essentially followed the method described by Okada, Murayama & Yamada (1966). A 0·5 ml sample of cells (2 × 10⁶/ml) in MEM containing 2 % calf serum was preincubated at 36 °C for 30 min with shaking under an aerobic condition and an equal vol. of the virus was then added to the cells. The mixtures were incubated at 36 °C for another 60 min with shaking. For the fusion of Vero cells in monolayer, coverslip cultures of Vero cells were prepared and infected following the method as described previously (Homma, 1971). At 3 h after infection, the monolayers were treated briefly with 0·02 % EDTA, fixed and stained with Giemsa solution (Kohn, 1965). Because we only had limited supplies of purified L Sendai virus, we could only use low input multiplicities of infection and there were accordingly no large multinucleated giant cells. For quantitative measurement we used the fusion index (FI) given by the equation:

\[ FI\% = \frac{\text{Total number of nuclei in fused cells}}{\text{Total number of nuclei}} \times 100. \]

Typical results are illustrated in Fig. 1, which reveals that the fusion activity of L Sendai both for Vero cells in monolayer and for Ehrlich's tumour cells in suspension was significantly restored by the trypsin treatment. Almost the same pattern was obtained irrespective of the...
Fig. 1. Restoration of the fusion activity of L Sendai by trypsin treatment. Test tubes containing L Sendai at $10 \times 2^8$ H.A.U./ml were treated with various concentrations of trypsin for 6 min at 36 °C. At the end of the incubation period, soybean trypsin inhibitor was added to the reaction mixtures to stop the enzymic action. The fusion activity was measured on Ehrlich's ascites tumour cells in suspension (●) and Vero cells in monolayer (○).

kind of cells and the cultural conditions used. Evidently, there was an optimal concentration of trypsin to induce the maximal enhancement of the fusion activity. It is clear from the results that apparent lack of the fusion activity with L Sendai is due to masking of the activity. It should be noted that the trypsin concentration which induced the maximal enhancement of the fusion activity was almost the same as that which induced the maximal enhancement of the haemolytic activity and of the infectivity for L cells (Homma, 1971, 1972), disclosing a close relationship among cell fusion, haemolysis and expression of the infectivity of Sendai virus.

Okada & Tadokoro (1962) found that treatment of Sendai virus with sonic vibration resulted in the increase of haemolytic activity, while it rather caused the decrease in fusion of the cells in suspension. Hosaka (1970) showed by electron microscopy that the size of the envelope fragments of Sendai virus responsible for haemolysis was the same as that for fusion in monolayer cells, but intact virus structure was required for fusion of the cells in suspension and for expression of the infectivity. As the present studies were confined to intact virus, haemolysis, cell fusion both in monolayer and in suspension, and infectivity for L cells are apparently expressed as the simultaneous events and the results do not seem to conflict with the above authors' finding.

Early fusion was also observed with NDV, a paramyxovirus related to Sendai virus (Johnson & Scott, 1964). No correlation of fusing ability with haemolytic activity was found among various strains of NDV (Kohn & Fuchs, 1969). Kohn (1965) also demonstrated
differences between haemolytic and fusion activities of NDV by treatment of the virus with freeze-thawing, heating and trypsin. Separation of haemolysin from Sendai virus and NDV by treatment with sodium deoxycholate was reported by Neurath (1964). It was uncertain, however, whether the isolated haemolysins were identical with those harbouring by these viruses. No data were available about fusion ability of the isolated haemolysins.

Host-dependent density differences in NDV were noted, as observed in the present system (Homma, 1971), by Stenback & Durand (1963) and Young & Ash (1970). The latter authors found that HeLa cell-passaged virus had a density higher than that of the original allantoic fluid virus and concomitantly lost the fusion activity. They have argued that the lipid which lowered the density of the virus particle was responsible for the virus-induced cell fusion. In this connexion, it should be emphasized that, in Sendai virus, whatever the nature of the fusion factor may be, the substance responsible for lowering the density is neither the principle for cell fusion nor the one for haemolysis. The trypsin treatment by which were induced both the haemolytic and fusion activities of L Sendai never affected the density of egg Sendai (1.174 g/ml) and L Sendai (1.207 g/ml) (Homma, 1971), supporting the view that the variation of Sendai virus in density and the one in both haemolysis and cell fusion are uncoupled host-controlled modifications.

It was shown that Sendai virus does not infect L cells because it is incapable of penetrating L cells (Homma, 1961). The present results show not only a close relationship between cell fusion and haemolytic processes of Sendai virus but also that these two activities are involved in the process by which Sendai virus penetrates into the cells. Morgan & Howe (1968) demonstrated that Sendai virus penetrates by fusion of the virus envelope with the host cell plasma membrane and release the nucleoprotein into the cytoplasm. In the light of this observation, the analysis of the masking and the unmasking of the infectivity for L cells as well as haemolytic and fusion activities of L Sendai may give an important clue for the study of the entry mechanism of Sendai virus.

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Department of Bacteriology
Tohoku University
School of Medicine
Sendai, Japan

REFERENCES

Short communications


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