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Trypsin Action on the Growth of Sendai Virus in Tissue Culture Cells

IV. Evidence for Activation of Sendai Virus by Cleavage of a Glycoprotein

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Results obtained by using a reconstitution technique on the Sendai virus envelope confirm that cleavage of one of the envelope glycoproteins (GP2) is prerequisite for activation of hemolytic and cell fusion activities of Sendai virus. The cleavage of GP2 occurs even when free envelope subunits are directly treated with trypsin in the presence of detergent. Trypsin treatment, either of the reconstituted particle or of the free envelope subunits but not of the intact virion, also causes a cleavage of the largest envelope glycoprotein (GP1), suggesting that a site on GP1 sensitive to trypsin becomes exposed during solubilization and reconstitution. The latter cleavage, however, is not associated with any change in biological activities.

Sendai viruses grown in various tissue culture cells were inactive in hemolysis, cell fusion, and infectivity as compared to egg-borne Sendai virus (egg Sendai) (1, 5, 9), and an attempt to activate them was achieved by in vitro treatment with low concentrations of trypsin (2, 3, 5). Polypeptide analysis of such inactive, trypsin-activated, and naturally active egg Sendai virions by polyacrylamide gel electrophoresis suggested that cleavage of one of the large glycoproteins (GP), GP2, was necessary for the activation of Sendai virus (4). Essentially the same suggestion has been made independently by Scheid and Choppin (12).

The experiments presented here were conducted to obtain more direct evidence in favor of the above supposition. To simplify the experimental system, reconstitution of the virus envelope (7, 8, 13) was exclusively used. The envelope particle reconstituted from egg Sendai after solubilization by Nonidet P-40 or Tween 20 was shown to retain all of the biological activities and the glycoprotein constituents carried by the virion envelope (7, 13). Using this technique, the biological properties of the reconstituted particles derived from either inactive or trypsin-activated virion and the effect of trypsin on the biological activity of such reconstituted particles were investigated in relation to the glycoprotein profiles on polyacrylamide gel electrophoresis. We used a culture of LLCMK2 cells, an established cell line of rhesus monkey kidney (15), for the growth of the Fushimi strain of Sendai virus (MK Sendai). This system gave an excellent virus yield with little contamination of cellular debris, and MK Sendai was shown to be least active in hemolysis and cell fusion among the viruses so far tested. Solubilization and reconstitution of the virus envelopes were performed by the method of Shimizu et al. (13). In brief, the virus envelope was solubilized with 0.5% Tween 20 (Emasol) in bicarbonate buffer, pH 10 (6), and the solubilized supernatant (after ultracentrifugation at 80,000 × g for 60 min) was applied to a column of Sephadex G-200 (25 by 360 mm) for removal of the detergent. The effluents were then collected in 3-ml fractions and allowed to stand for 24 h at room temperature to reconstitute the envelope. At the end of this period, each fraction was assayed for hemagglutinating, neuraminidase, and hemolytic activities as described elsewhere (3, 17). Since hemolytic and cell fusion activities of Sendai virus were shown to correspond with each other (3, 5), only hemolytic activity was measured as an indication of biological activities.

The results obtained are shown in Fig. 1a. Fractions 8 to 13 showed hemagglutinating and neuraminidase activities but no hemolytic activity. When such fractions were treated with trypsin (3), hemolytic activity became manifested, whereas the others remained unchanged (Fig. 1b). The results demonstrate that the inactive virion also yielded the inactive reconstituted particle, which could also be activated by trypsin. The subsequent experiment was designed to show the direct effect of trypsin

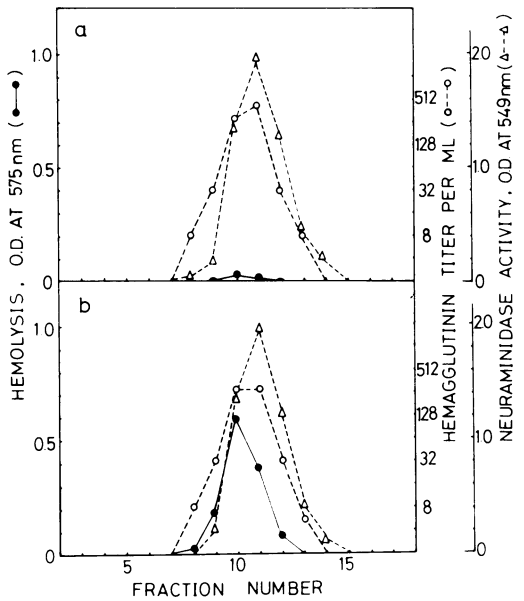

FIG. 1. *Gel filtration profiles of the solubilized envelopes of MK Sendai virus through a Sephadex G-200 column. MK Sendai virion (5,000 hemagglutinating units) was treated with 0.5% Tween 20 at 20 C for 1 h in 2 ml of bicarbonate buffer, pH 10. After centrifugation at 80,000 × g for 60 min the solubilized supernatant was applied to a column of Sephadex G-200 (25 by 360 mm) equilibrated with phosphate-buffered saline, pH 7.2 (13). A 3-ml aliquot eluted by phosphate-buffered saline was collected in a test tube and allowed to stand for 24 h at room temperature. Each fraction was divided into two portions and assayed for hemagglutinin (○), neuraminidase (△), and hemolytic activities (●) before (a) and after (b) trypsin treatment. Trypsin treatment was performed as described in a previous paper (3).*

on the free envelope subunits of the inactive MK Sendai virus. MK Sendai was solubilized and clarified by centrifugation as described. The supernatant fluid that was known to contain exclusively free envelope subunits (13) was treated directly with trypsin at 0.002% for 6 min at 20 C in bicarbonate buffer, pH 10, and then subjected to the Sephadex column. The same fractionation pattern as Fig. 1b was obtained, suggesting that trypsin might act directly on the free envelope subunits responsible for hemolysis.

The above results were directly compared to molecular events by polypeptide analysis of the reconstituted particle obtained from the mixture of MK Sendai virions separately labeled with L-[^{3}H]leucine and D-[^{14}C]glucosamine on polyacrylamide gel electrophoresis (4). As expected, the inactive reconstituted particle consisted of two major glycoproteins, GP1 and GP2, and one minor one, GP3 (Fig. 2a); the glycoprotein pattern is characteristic of the inactive Sendai virion (4). On the other hand, a pattern entirely different from the above one was obtained when the inactive reconstituted particle was activated by trypsin (Fig. 2b). The radioactive counts of GP2 were completely removed, and the counts of GP3 and GP4 increased significantly. In regard to these glycoproteins, the same results were obtained previously when the intact virion was activated by trypsin, supporting the idea that trypsin cleaves GP2 (4). In addition, it was unexpectedly found that the activation of the reconstituted particle by trypsin also caused a cleavage of GP1, which was accompanied by a concomitant appearance of GP1c at a position located between GP2 and GP3 and another glycoprotein migrating rather heterogeneously between GP3 and GP4 (Fig. 2b). The same cleavage phenomenon has been noticed during solubilization of the envelope of egg Sendai (Tozawa, personal communication), which is known to lack GP2 (4). Almost the same pattern as seen in Fig. 2b was obtained with the reconstituted particle whose free envelope subunits had been directly treated with trypsin as described. The cleavage of GP1 was not observed, however, with the particle reconstituted from trypsin-activated MK Sendai virion. This particle consisted of GP1, GP3, and GP4.

The overall results can be summarized as follows. (i) The inactive virion yielded the inactive reconstituted particle with the uncleaved form of GP2, and the virion activated by trypsin yielded the active reconstituted particle with the cleaved form of GP2. (ii) The inactive reconstituted particle could be activated by trypsin, accompanied by the cleavage of GP2. (iii) Treatment of the free envelope subunits with trypsin yielded the active reconstituted particle with the cleavage of GP2. These results lead to the conclusion that the cleavage of GP2 is caused by direct action of trypsin and that this step is prerequisite for the activation of Sendai virus. From this point of view, an idea previously proposed by one of us (M. H.), that biologically inactive Sendai virus would have an inhibitory substance from the host cells (2, 3), is unlikely and should be discarded.

The cleavage of GP2 into GP3 and GP4 was already assumed in our previous paper (4). GP4 is such a rapidly moving minor component that it is hardly detectable with the stained gels, but it can be detected when isotopically labeled virions are analyzed. It was recently shown by analysis of the labeled preparation that even egg Sendai had GP4 together with GP3 (14). Since the molecular weights of GP2, GP3, and

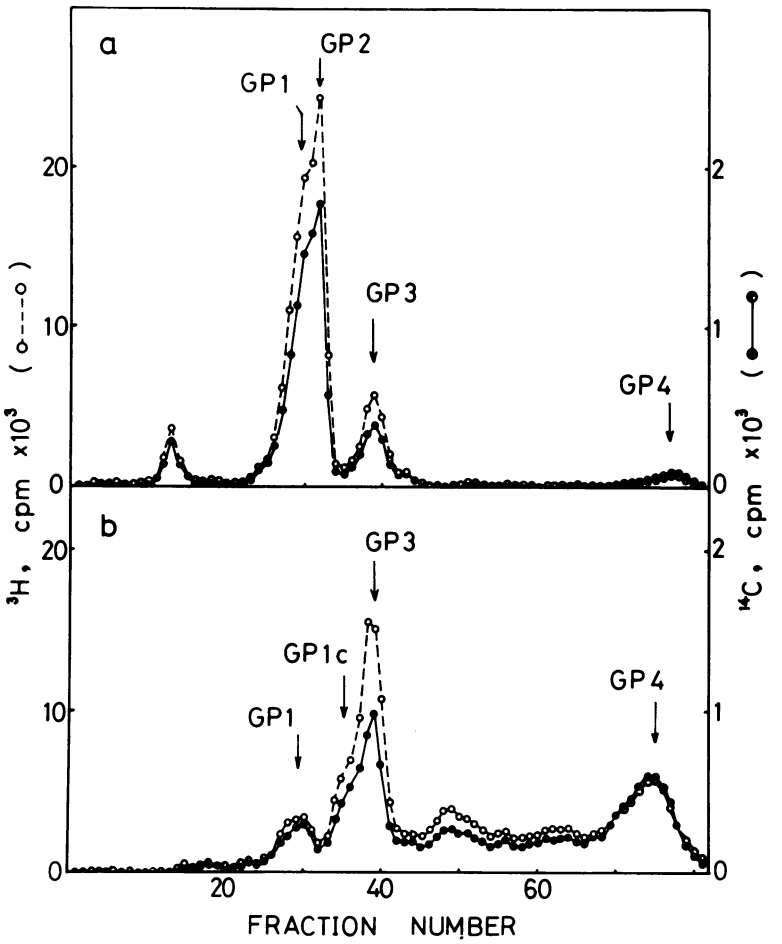

FIG. 2. *Electrophoretic patterns of radioisotope-labeled reconstituted particles before and after treatment with trypsin. The mixture of MK Sendai virions labeled separately with* L-[^{3}H]*leucine and* D-[^{14}C]*glucosamine was solubilized and filtered through a Sephadex G-200 column and reconstituted as described in the legend for Fig. 1. Fractions 9 to 11, having hemagglutinating activity, were pooled and divided into two groups. One-half was treated with 0.0004% trypsin (b), and the other half was mock-treated with phosphate-buffered saline (a). The particles in each group were collected by precipitation with trichloroacetic acid at a final concentration of 6%. After removal of trichloroacetic acid by ethanol and ether, the precipitates were subjected to polyacrylamide gel electrophoresis as described in the previous paper (4). After the run, each gel was sliced (0.7 mm thick), and the radioactivity of each disk was measured.*

GP4 have been shown to be 65,000, 51,000, and 15,000, respectively (14), GP2 might be composed of one molecule each of GP3 and GP4.

Unexpectedly, the present experiments have revealed that the cleavage of GP1, which carries both HA and neuraminidase activities (12, 14, 16), could occur only when trypsin treatment was made on the reconstituted particle or on the free envelope subunits. This cleavage, however, never occurred with the intact virion, even after treatment with trypsin, suggesting that the site of GP1 sensitivity to trypsin may be buried in the envelope with the intact virion. The cleavage of GP1 was not accompanied by any change of hemagglutinating and neuraminidase activities as well as hemolytic activity, and the phenomenon could well be compared with the cleavage of influenza virus HA (10, 11).

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