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NOTES

Inactivation of Polykaryocytogenic and Hemolytic Activities of Sendai Virus by Phospholipase B (Lysolecithinase)

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Evidence is presented that lysolecithin is involved in the fusing and hemolytic activities of Sendai virus. Treatment of the virus with phospholipase B (lysolecithinase) specifically inactivates the hemolytic and fusing abilities, without affecting the infectivity and the capability of the virus to hemagglutinate and adsorb to cells. The possible identity of lysolecithin with the "cell fusion factor" of paramyxoviruses and the mechanism of cell fusion are discussed.

Viruses of the paramyxovirus group induce cell fusion by a mechanism which is still unknown, although a "cell fusion factor" distinct from the viral hemagglutinin and unrelated to infectivity (4) is present in the viral envelope. Recently, cell fusion was performed by treating cells in suspension with a solution of purified lysolecithin (8), and the good yield of viable polykaryocytes obtained by this procedure (C. Croce et al., Exp. Cell Res., *in press*) suggests that this technique may artificially recreate a physiological mechanism of fusion. Because the structural analysis of paramyxoviruses demonstrates that they contain lysolecithin in the envelope (2, 9), one can postulate that lysolecithin is related to the "cell fusion factor." We tested this hypothesis by treating Sendai virus with phospholipase B (lysolecithinase) and assayed the effect of this treatment on the fusing, hemagglutinating, and hemolytic activities and on the infectivity of the virus.

A pool of Sendai virus was prepared by infecting 9-day-old embryonated chicken eggs. The virus was concentrated by high-speed centrifugation and inactivated with β-propiolactone by a technique described previously (1). The concentrated viral preparation contained 40,000 hemagglutinating units (HAU) per ml.

Treatment with lysolecithinase (Phospholipase B, Pierce Chemical Co., Rockford, Ill.) was performed by adding 1 ml of 0.2 M phosphate buffer (*p*H 6) and 0.2 mg of lysolecithinase, suspended in 1 ml of the same buffer, to 1 ml of concentrated Sendai virus. (Phospholipase B produced only one band when tested by polyacrylamide gel electrophoresis, indicating a satisfactory purity of the enzyme. The lysolecithinase activity of the enzyme was confirmed by its capacity to inactivate the fusing and hemolytic activities of purified lysolecithin.) This mixture was incubated for 30 min at room temperature and then centrifuged at 47,000 $\times$ *g* for 15 min in the no. 30 rotor of an ultracentrifuge (Spinco model L). The supernatant fraction was discarded, and the pellet was resuspended in 1 ml of phosphate buffer. Control virus was treated in the same way, except that 1 ml of phosphate buffer was added in place of lysolecithinase.

Fusion was carried out by mixing 5 $\times$ 10^6 KB cells with 4,000 or 2,000 HAU of Sendai virus, following a technique described elsewhere (1). The acid *p*H, created in the fusion mixture by the addition of virus suspended in phosphate buffer at *p*H 6, was brought to neutrality with a few drops of 0.1 M NaOH.

The fusion experiments indicate that the virus treated with lysolecithinase lost fusing ability almost completely (Fig. 1) as determined by the number of polykaryocytes and the fusion index (Table 1). The hemolysin of the virus was also destroyed by the treatment with lysolecithinase (Fig. 2 and Table 1).

Hemagglutinating activity was not altered, since the enzyme-treated virus had the same hemagglutinating titer for guinea pig red blood cells as the control virus (Table 1).

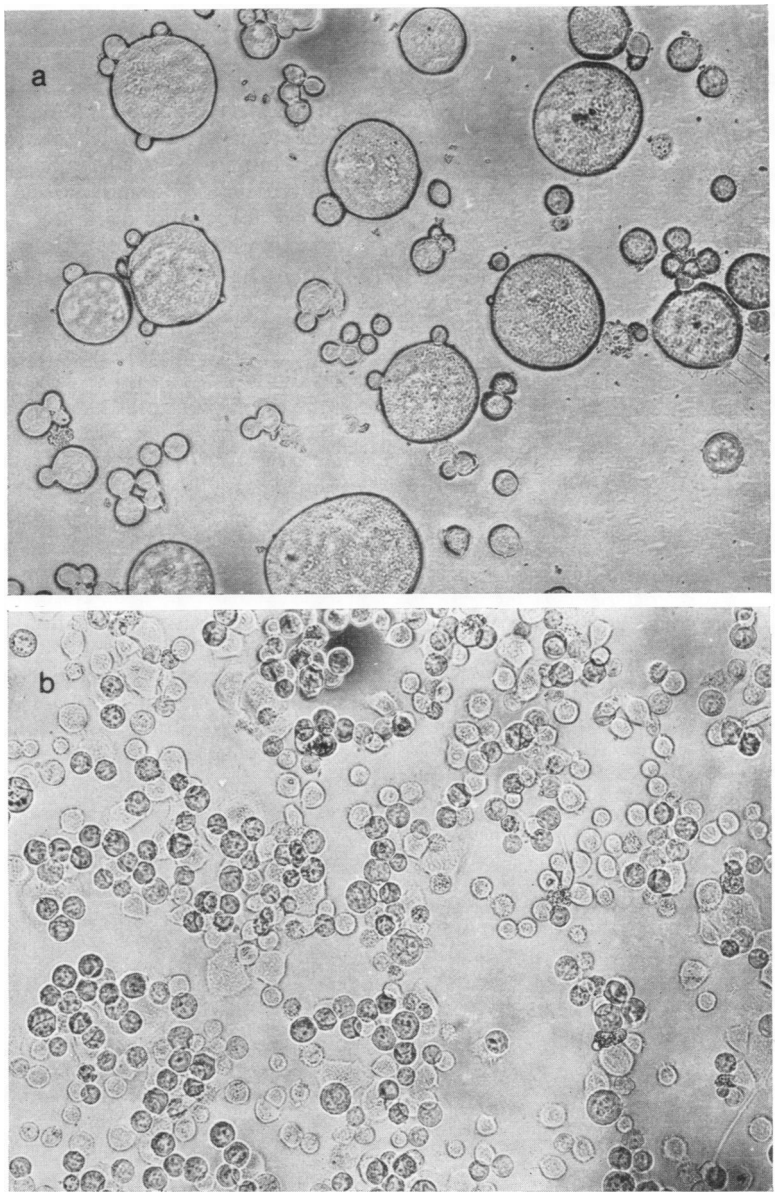

FIG. 1. *Photograph taken 1 hr after fusion of 5 × 10⁶ KB cells with 4,000 hemagglutinating units of control Sendai virus (a) and of Sendai virus treated with lysolecithinase (b). × 300.*

TABLE 1. *Effect of lysolecithinase on various properties of Sendai virus*

Treatment	Fusion				Hemolysin (% activity remaining)	Hemagglutinin (% activity remaining)	Infectivity (log $TCID_{50}$/0.1 ml)
	Polykaryocytes (%)		Fusion index[a]				
	Expt 1[b]	Expt 2[c]	Expt 1	Expt 2			
SPB[d]	45.2	16.1	4.05	2.96	100	100	6.5
SL[e]	2.98	0.91	1.2	1.1	0.25	100	6.5
Uninfected KB cells	0.93	0.89	1.1	1.1			

[a] Fusion index represents the mean number of nuclei per cell. It is expressed as the ratio of total number of nuclei to the number of cells in which these nuclei were counted.

[b] KB cells (5×10^6) were fused with 4,000 hemagglutinating units (HAU) of Sendai virus.

[c] KB cells (5×10^6) were fused with 2,000 HAU of Sendai virus.

[d] Control Sendai virus treated with phosphate buffer.

[e] Sendai virus treated with lysolecithinase.

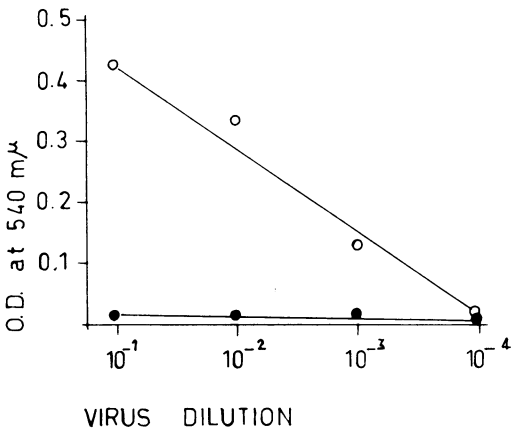

FIG. 2. *Hemolytic activity of control Sendai virus and Sendai virus treated with lysolecithinase. The hemolytic activity was determined by mixing serial 10-fold dilutions of concentrated Sendai virus with guinea pig red blood cells (concentration 0.5% in phosphate-buffered saline). After 4 hr of incubation at 4 C, the tubes were put in a water bath at 37 C and shaken every 30 min for 2 hr. The amount of hemoglobin released in the supernatant fluid was then measured by reading the optical density at 540 nm. Symbols:* (○) *control Sendai virus;* (●) *Sendai virus treated with lysolecithinase.*

The capability of adsorbing to cells was also unaffected or even enhanced in some experiments, because the amount of virus recovered after fusion in the supernatant fluid of cells fused with lysolecithinase-treated Sendai virus was equal to or lower than the amount of virus recovered in the supernatant fluid of cells fused with control Sendai virus.

The infectivity of the virus, determined by the appearance of syncytia and cytopathic effect in infected KB cells, was unchanged after treatment with lysolecithinase (Table 1).

Electron microscopic observation, by negative staining of the lysolecithinase-treated virus, showed an increase in filamentous forms (Fig. 3a, b) and a modification of the outer layer in the viral envelope (Fig. 4a, b). Strong alteration or disruption of the viral particles was seldom observed.

The above results demonstrate that treatment with lysolecithinase does not cause a drastic alteration of the virus as indicated by its ultrastructural appearance and by the persistence of hemagglutinating activity and infectivity.

On the other hand, the enzymatic treatment selectively inactivates the fusing and hemolytic abilities. This finding suggests that the two viral properties are correlated, and lysolecithin is a mediator of both fusing and hemolytic activities.

Indeed, a remarkable parallelism was found, on the basis of several characteristics, between hemolysin and the "cell fusion factor" of paramyxoviruses (6). Recently, Hosaka showed that, after fractionation of disrupted Sendai virus in a sucrose gradient, the fusion activity had a distribution range similar to the hemolytic activity (5). Moreover, the inactivation of the hemolysin and the "cell fusion factor" by treatment either with ether (10) or with lipolytic enzymes (6) supports the idea that a lipid component is essential for the display of both hemolytic and cell-fusing abilities, even though viral protein(s) may play some role in hemolysis. In fact, proteolytic enzymes partially inactivate the hemolysin without affecting the "cell fusion factor" (6, 7).

The evidence provided by Elsbach et al. (3) that mammalian cells contain lysolecithinase activity and are able to convert lysolecithin to lecithin suggests an explanation for the mechanism of cell fusion induced by paramyxoviruses. In fact, the cell could regulate the lytic activity of lysolecithin contained in the viral envelope so that the interaction of a syncytiogenic virus with cells would end in cell fusion and not in cell lysis.

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LITERATURE CITED

1. Barbanti-Brodano, G., S. Oyanagi, M. Katz, and H. Koprowski. 1970. Presence of two different viral agents in brain cells of patients with subacute sclerosing panencephalitis. Proc. Soc. Exp. Biol. Med. **134**:230–236.
2. Blough, H. A., and D. E. M. Lawson. 1968. The lipids of paramyxoviruses: a comparative study of Sendai and Newcastle disease viruses. Virology **36**:286–292.
3. Elsbach, P., K. V. Holmes, and P. W. Choppin. 1969. Metabolism of lecithin and virus-induced cell fusion. Proc. Soc. Exp. Biol. Med. **130**:903–908.
4. Guggenheim, M. A., S. Tyrrell, and A. S. Rabson. 1968. Studies on Sendai virus cell fusion factor. Proc. Soc. Exp. Biol. Med. **129**:854–857.
5. Hosaka, Y. 1970. Early cell fusion by sonicated H.V.J., p. 684–696. *In* R. D. Barry and B. W. J. Mahy (ed.) The biology of large RNA viruses. Academic Press Inc., New York.
6. Kohn, A. 1965. Polykaryocytosis induced by Newcastle disease virus in monolayers of animal cells. Virology **26**:228–245.
7. Neurath, A. R. 1963. Effect of trypsin treatment on the hemolytic, hemagglutinating, neuraminidase and adenosine diphosphatase activities of Sendai virus. Acta Virol. (Prague) **7**:490–497.
8. Poole, A. R., J. I. Howell, and J. A. Lucy. 1970. Lysolecithin and cell fusion. Nature (London) **227**:810–813.
9. Rebel, G., R. Fontanges, and L. Colobert. 1962. Nature lipidique des substances responsables de l'activité hémolytique de myxovirus parainfluenzae 1 (virus Sendai). Ann. Inst. Pasteur **102**:137–152.
10. Sokol, F., D. Blaskovic, and O. Krizanova. 1961. Subunits of myxoviruses. II. Properties of hemagglutinins of Newcastle disease, parainfluenza 1 and mumps viruses. Acta Virol. (Prague) **5**:153–159.

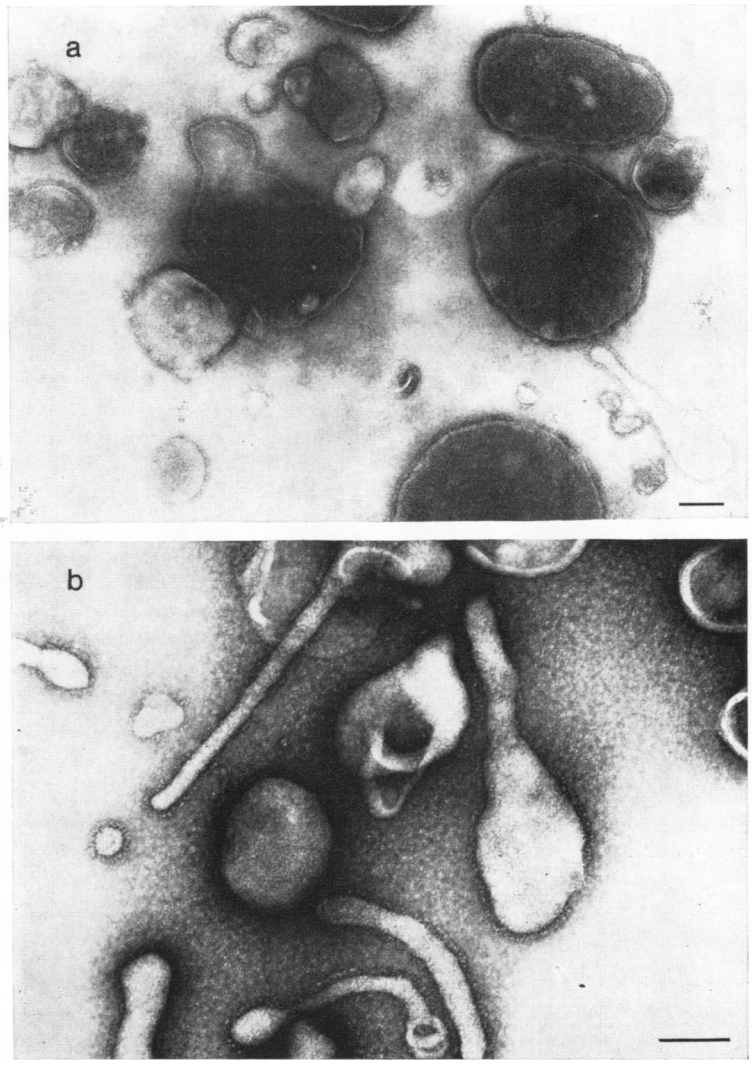

FIG. 3. *Negative staining with phosphotungstic acid of control Sendai virus (a) and Sendai virus treated with lysolecithinase (b). Note the prominence of filamentous forms in the enzyme-treated virus. Bars represent 0.1 μm.*

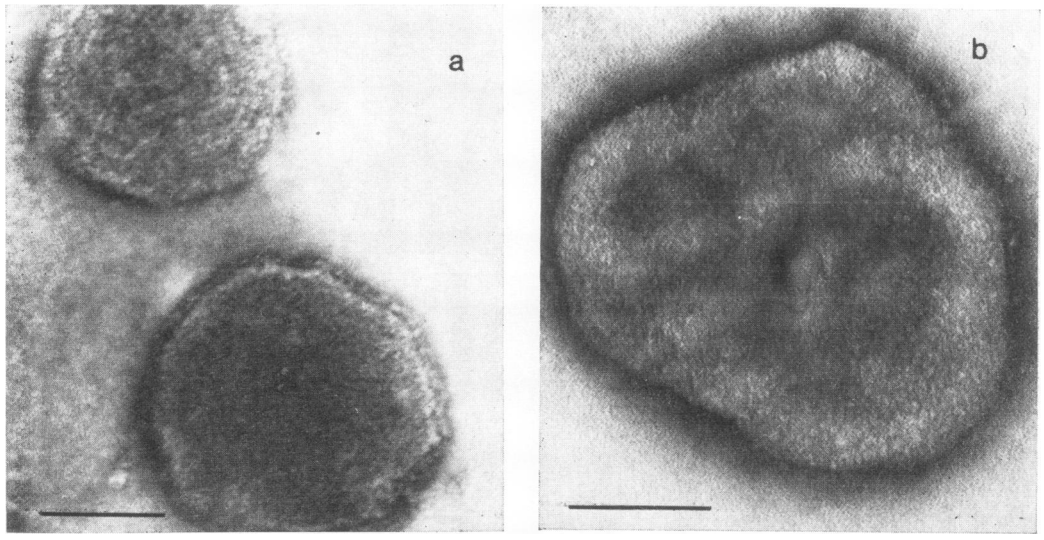

FIG. 4. *High magnification of control Sendai virus (a) and Sendai virus treated with lysolecithinase (b). Note the fluffy appearance of the outer coat in the enzyme-treated virus. Bars represent 0.1 μm.*