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SHORT COMMUNICATION

RIBONUCLEASE-SENSITIVE INFECTIVITY OF CHIKUNGUNYA VIRUS FROM BHK21 CELLS INFECTED WITH THE VIRUS

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During studies on an intracellular component associated with Chikungunya virus (ChV)-specific RNA, it was found that the virus infectivity (PFU) in a homogenate of infected cells sedimented faster than the PFU in the infected fluid on sucrose gradient analysis (Igarashi and Fukai, 1969). This faster sedimentation of the ChV-PFU in the cell homogenate was thought to result from association of the ChV-PFU with some cellular components. Like other group A arboviruses (Morgan, Howe and Rose, 1961; Mussgay and Weibel, 1962; Acheson and Tamm, 1967), ChV matures by budding of the nucleoprotein core through the infected cell membrane (Higashi et al., 1967), so if infected cells are homogenized, the budding virus structures will be released in association with some cellular components and may constitute the faster sedimenting infectivity. If so, some of the particles will be devoid of part of the outer membrane and their PFU may be ribonuclease (RNase)-sensitive, because the RNA in the nucleoid structure of ChV (Igarashi, Fukuoka and Fukai, 1969) is sensitive to RNase (Igarashi and Fukai, 1969).

Chikungunya virus (ChV), African strain, was used. The methods used to obtain seed virus, to infect and label the cells with ^{3}H-uridine in the presence of Actinomycin S_3, and to prepare a cell homogenate have been described (Igarashi and Fukai, 1969; Igarashi, 1969). The host cells used for virus multiplication were a baby-hamster kidney-cell line, clone 13 (MacPherson and Stoker, 1962), which was cultivated by the method of Karabatsos and Buckley (1967). ChV-infectivity (PFU) was assayed by plaque titration on an established cell line of African green monkey kidney (VERO) as described before (Igarashi and Tuchinda, 1967). Crystalline ribonuclease (RNase) was a product of Worthington Biochemicals Co. Uridine-5-^{3}H (5 c/mmole) was purchased from Daiichi Pure Chemicals Co. Tokyo. Sucrose gradient analysis and counting the acid-insoluble radioactivity were performed as described previously (Igarashi and Fukai, 1969). Antiserum against ChV was prepared in adult mice (Igarashi, 1969) and was fractionated by passing a column of Sephadex G-200 to get globulin fraction.

BHK21 cells were harvested 16 hr after infection with ChV. The fluid and cell homogenate of the infected culture were fractionated by sucrose gradient sedimentation. ChV-PFU in each fraction was assayed after incubation with an equal volume of H_2O or RNase. The results in Fig. 1 indicate that the ChV-PFU from the infected fluid sedimented to fraction 5 and was resistant to RNase, while the ChV-PFU from the infected cell homogenate sedimented to fraction 3 and was partially

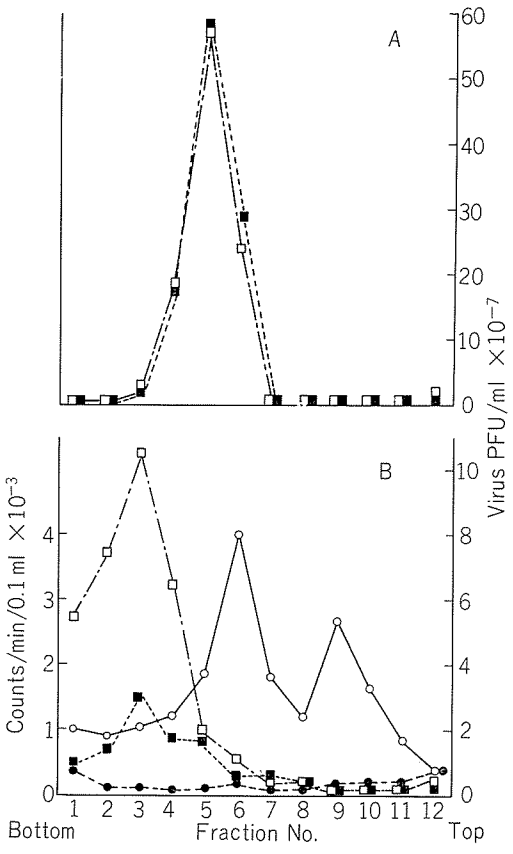

FIGURE 1. *Sucrose gradient analysis of a homogenate of ChV-infected BHK21 cells and the culture fluid.*

Monolayer cultures of BHK21 cells (1.5×10^7 cells) were infected with ChV at an input multiplicity of 20. After adsorption at 37C for 2 hr, cell sheets were washed twice with PBS (Dulbecco and Vogt, 1954) and minimal essential medium of Eagle (1959) containing 1 μg/ml of Actinomycin S_3 was added. The cell sheets were incubated at 37 C for 2 hr. Then ^{3}H-uridine was added at a final concentration of 5 μc/ml, and incubation at 37 C was continued. At 16 hr after infection, infected fluids were removed and centrifuged at 700×g for 10 min. The supernatant was fractionated by sucrose gradient sedimentation (10–35% gradient in 0.1M NaCl 0.02M Tris-HCl, 0.001M EDTA, pH 7.6, 30,000 rev/min, 60 min, SW-39 rotor of a Beckman model L ultracentrifuge). Each fraction was assayed for its ChV-PFU after incubation with an equal volume of H_2O (□- - - -□) or of 20 μg/ml of RNase (■·····■) at 37 C for 30 min in (A). Infected cell sheets were washed twice with PBS and homogenized in 1 ml of 0.1M NaCl, 0.02M Tris-HCl, 0.001M EDTA, pH 7.6, and centrifuged at 700×g for 10 min. The supernatant was analyzed as described above. Acid-insoluble radioactivity after incubation with H_2O (○———○) or with RNase (●·····●) is also shown in (B).

sensitive to RNase. Neither RNase-resistance nor the position in the sucrose gradient of the ChV-PFU were affected when the infected fluid was homogenized before sucrose gradient analysis. When fraction 5 of Fig. 1A and fraction 3 of Fig. 1B were incubated with globulin fraction of anti-ChV serum (37 C, 2 hr), the PFU in both specimens were neutralized to the same extent. Fraction 3 of Fig. 1B was analyzed by CsCl isopycnic density gradient sedimentation (15–40% preformed gradient, 50,000 rev/min, 2 hr, SW-65L rotor of a Beckman model L2-65B ultracentrifuge). The peak of acid-insoluble radioactivity was found at a density of 1.19 g/cc, which was less than that of partially purified ChV (1.24 g/cc). This may be the result of association of the former with some less dense cellular components.

The existence of RNase-sensitive PFU was examined at different stages of ChV-infection. The cell homogenate and fraction 3 obtained by centrifugation of it in a sucrose gradient were assayed for ChV-PFU after incubation with H_2O or RNase. The PFU titer of the cell homogenate after incubation with RNase was always a little lower than that after incubation with H_2O, while the two were the same with infected fluid (Table 1). The ratio of PFU after incubation with RNase to PFU after incubation with H_2O tended to decrease at a later stage of infection, suggesting that both the absolute amount, and RNase-sensitive fraction, increased at the late stage of infection. This seems interesting in relation to the results obtained with an intracellular component associated with ChV-RNA. That is, even when the release of mature ChV ceased at the late stage of infection, there was an appreciable amount of ChV-specific ^{3}H-uridine incorpo-

TABLE 1. *RNase-sensitive PFU in ChV-infected BHK21 cell homogenate.*

Hours after infection	Cell homogenate supernatant before fractionation			Fraction 3 of sucrose gradient analysis of cell homogenate		
	Virus PFU/ml after incubation with		Ratio of PFU in RNase to H_2O	Virus PFU/ml after incubation with		Ratio of PFU in RNase to H_2O
	H_2O	RNase		H_2O	RNase	
2	8.7×10^5	4.0×10^4	0.46	1.1×10^5	8.5×10^4	0.77
4	1.3×10^7	9.4×10^6	0.73	7.0×10^5	4.1×10^5	0.58
6	1.0×10^8	6.2×10^7	0.62	1.5×10^6	1.2×10^6	0.80
8	1.0×18^8	6.5×10^7	0.65	7.5×10^6	5.5×10^6	0.74
10	1.5×10^8	7.0×10^7	0.47	7.5×10^6	4.7×10^6	0.63
12	2.9×10^8	8.0×10^7	0.28	7.5×10^6	4.0×10^6	0.54
16 (fluid)	1.0×10^9	1.1×10^9	1.08			

Replicate cultures of BHK21 cells (7×10^6 cells/culture) were infected with ChV as described in the lenged of Fig. 1. At 2 hr intervals after infection, 2 replicate cultures were harvested. Cell homogenates were prepared and fractionated by sucrose gradient sedimentation as is Fig. 1. A part of the supernatant of the cell homogenate and fraction 3 obtained by sucrose gradient analysis were incubated with an equal volume of H_2O or RNase (20 μg/ml) at 37 C for 30 min. ChV-PFU in each specimen was assayed.

ration, possibly into nucleoid structures in the infected cells, suggesting that the pool size of this nucleoid was expanding, or that there was a blockage of some maturation step (Igarashi and Fukai, 1969). The RNase-sensitive fraction of ChV-PFU associated with infected cells may represent budding particles. In a pulse-chase experiment with ^{3}H-uridine at an early stage of ChV-infection, a small peak of ^{3}H-activity was transiently observed in fraction 3 (Igarashi and Fukai, 1969). These particles, have a part necessary for infection, which is neutralized with antiserum, but still have an incomplete outer membrane, enabling RNase to attack the nucleoprotein core. RNase-sensitive particles have also been found in the growth cycle of avian myeloblastosis virus (Allen, 1966), which also matures by budding.

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