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RELATIONSHIP OF 2', 3'-CYCLIC NUCLEOTIDE 3'-PHOSPHOHYDROLASE ACTIVITY OF LARGE ENVELOPED RNA VIRUSES TO HOST CELL ACTIVITY

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Summary. - Purified virions of the large RNA viruses show 2', 3'-cyclic nucleotide 3'-phosphohydrolase (3'-CNPase) activity. The 3'-CNPase activity is virion-associated and stimulated by their treatment with nonionic detergents. Cytopathic viruses such as influenza A2 (Singapore/57), NDV, and VSV showed the specific activity of a virion-associated 3'-CNPase equal to or lower than the specific activity of host cell enzyme. Retroviruses are an example of extreme relationship of 3'-CNPase to virion. With the AMV-BAI-A associated enzyme activity increased after Triton X-100 treatment ten times more than that associated with other viruses examined. The specific activity of virus-associated 3'-CNPase was 16–28 times higher than that in chick myeloblasts. BLV showed a very low enzyme activity. The correlation between the activity of cellular 3'-CNPase and virus yield showed that 3'-CNPase could belong to cellular factors influencing virus replication.

Key words: 2', 3'-cyclic nucleotide 3'-phosphohydrolase; large RNA viruses; host cells

Introduction

Various animal cells contain 2', 3'-cyclic nucleotide 3'-phosphohydrolase (3'-CNPase) which converts ribonucleoside 2', 3'-cyclic phosphates to 2'-ribonucleosidmonophosphates (2'-NMP). 3'-CNPase has been intensively studied first of all in neurons (Trapp *et al.*, 1988). The enzyme was isolated from neural tissue, purified and its properties were determined (EC 3. 1. 4. 37). In addition to other cell systems (Lees *et al.* , 1974; Dreiling *et al.* , 1981; Pristašová *et al.* , 1981), we found the enzyme associated with myxo- and paramyxoviruses (Rosenbergová *et al.* , 1981). On the example of NDV we showed that the enzyme was bound to the nucleocapsid (Rosenbergová and Pristašová, 1990), possibly having been acquired from host cells. We do not know its func-

tion neither in the cell nor during virus replication. We decided to find out whether 3'-CNPase was associated with other large RNA viruses and whether virus replication could influence the cellular 3'-CNPase activity.

Materials and Methods

Viruses. Newcastle disease virus (NDV) strain Kansas, influenza virus A2/Singapore/57(H2N2), vesicular stomatitis virus (VSV) strain Indiana were grown in 10-day-old chick embryos for 46–48 hr at 35 °C. The viruses were purified from allantoic fluid by differential centrifugation in discontinuous and continuous density gradient (Rosenbergová *et al.*, 1981). VSV was grown in Vero cells (10^8–10^9 PFU/ml) and then adapted to CCL64, L and chick embryo cells (CEC). After centrifugations at 10,000 xg (15 min) and 40,000 xg (60 min) the sediment was resuspended in 0.1 mol/l Tris-HCl pH 7.2 and separated in discontinuous 20–30 % sucrose gradient at 182,000 xg. The viral band was dissolved in 0.1 mol/l Tris-HCl pH 7.2, sedimented at 182,000 xg for 30 min and resuspended in the same buffer. Avian myeloblastosis virus (AMV) strain BAI-A was prepared by centrifugation of leukaemic chicken plasma (5 times diluted in 0.01 mol/l phosphate buffer pH 7.2) at 33,000 xg for 45 min and purified by separation in discontinuous sucrose gradient (10–50 %) at 140,000 xg for 4 hr. Bovine leukaemia virus (BLV) was kindly provided by Dr. Č. Altaner (Institute of Experimental Oncology, Slovak Academy of Sciences, Bratislava).

Viral suspensions were used as enzyme source at protein concentration of 2 mg/ml in 0.05 mol/l Tris-HCl buffer pH 7.2 in the presence of 0.01 % natrium azide.

Cells. CEC were prepared by trypsinization of 11-day-old CE (Dulbecco and Vogt, 1954); mink lung cells (CCL64) were a kind gift of Dr. J. Závada (Institute of Virology, Bratislava); foetal lamb kidney cells (FLK-GI/II) were provided by Dr. Č. Altaner (Institute of Experimental Oncology, Bratislava); L (normal subcutaneous areolar and adipose mouse tissue cells) and Vero (African green monkey kidney cells) were obtained from the stale of our Institute. All cells were propagated in BEM containing 10 % inactivated calf serum.

The enzymic activity was determined in cell extracts prepared in 0.001 mol/l Tris-HCl buffer pH 7.2 in deeply cold ethanol - dry ice by 3 times freeze-thawing and subsequent centrifugation (19,000 xg, 90 min). Supernatants were adjusted to protein concentration 2mg/ml in 0.05 mol/l Tris-HCl pH 7.2 and used as enzyme source.

Enzymic reactions. The enzymic activity was tested using 2', 3'-guanosin cyclic phosphate as substrate. The reaction mixture contained 100 μg substrate, 10–200 μg viral protein or 20–250 μg cellular protein in a total vol of 200 μl in presence of 0,05 mol/l Tris-HCl pH 7.2 After incubation for 2 hr at 30 °C the reaction was stopped by dipping into boiling water bath. The proteins were removed by extraction with 2–3 vol of chloroform. The reaction product 2'-guanosin monophosphate was determined by paper chromatography (Rosenbergová and Pristašová, 1990).

VSV replication and 3'-CNPase synthesis kinetics were compared in L and Vero cells. Cell monolayers grown in Petri dishes were overlaid with 1 ml virus in BEM supplemented with 10 % ITS (10 PFU/cells). After 1 hr adsorption the inoculum was removed, the cells were washed with phosphate buffered saline (PBS) pH 7.2, replenished with fresh BEM and further incubated at 37 °C. Virus infectivity was tested at 0, 2, 4, 6, 8, 10, 12, 16, 20, and 24 hr post-infection (p.i.) in the total mixture of cells and culture fluid prepared by 3 times freezing and thawing on dry ice-cooled ethanol.

VSV titration was performed on CEC. The viral samples were diluted in PBS containing 1 % foetal calf serum and adsorbed at room temperature for 90 min. After washing the cells were overlaid with 0.9 % agar in Earle's solution containing 1 % ITS. After 48 hr incubation at 37 °C the plaques were stained with 1 % neutral red. Virus titre was expressed in PFU/ml.

To detect the enzymic activity of infected cells, they were sedimented 15 min at 150 xg and treated with 1 % Triton X-100 in 0.001 mol/l Tris-HCl pH 7.2. After three times freezing and thawing and centrifugation at 1,000 xg the supernatant was used to determine the enzyme activity.

Results and Discussion

Table 1 summarizes the results of experiments in which the activity of 3'-CNPase associated with large RNA virions was compared with that of host cells. From cytopathogenic viruses three representatives were selected: influenza virus (orthomyxovirus), NDV (paramyxovirus), and vesicular stoma-

Table 1. Comparison of the activity of 2', 3'-cyclic nucleotide 3'-phosphohydrolase in large RNA viruses and in host cell species

Cells and viruses		Activity of 3'-CNPase		Enzyme stimulation by Triton	*Relative activity
		Triton$^-$	Triton$^+$		
Cells:	CEC	2.68	2.74	1.02	1.00
Virus:	Influenza	0.65	2.0	3.1	0.73
	NDV	0.45	2.10	4.6	0.76
	VSV	1.25	2.79	2.7	1.01
Cells:	CCL64	8.6	7.6	0.88	1.00
Virus:	VSV	1.30	2.60	2.0	0.34
Cells:	L	0.9	0.85	0.94	1.00
Virus:	VSV	0.2	0.7	3.5	0.82
Cells:	Vero	13.0	14.0	1.08	1.00
Virus:	VSV	3.4	6.55	1.9	0.46
Cells:	FLK-GI/II lamb kidney	0.7	0.4	0.57	1.00
Virus:	BLV	0.3	0.0	0.0	0.0
Cells:	Chick myeloblasts	4.8	4.9	1.02	1.00
Virus:	AMV I.	1.6	77.5	48.4	15.8
	AMV II.	1.8	136.0	75.6	27.8

Enzyme assay is described in *Materials and Methods.* Enzyme activity is expressed as A_{260} nm of 2'-GMP produced by 1.0 mg of viral or cell protein

*Relative activity is expressed as ratio of enzyme activity of the virus to enzyme activity of respective uninfected cells (except of chick myeloblasts and FLK-GI/II cells). The index was calculated from activities estimated in the presence of 1 % Triton X-100.

titis virus (VSV) (rhabdovirus). All three viruses grown in CEC had low enzymic activity, which increased in the presence of 1 % Triton X-100 by approximately same rate (2.0–2.8 times). In comparison with the enzyme activity of host cells (CEC), the relative virus-associated activity was equal or lower. In CEC primocultures the examined viruses did not concentrate 3'-CNPase activity. The results showed that enzyme activity had been acquired during virus maturation from the host cell and that the amount of acquired enzyme activity was in correlation to the host cell-associated enzyme activity.

Because VSV has a broad host cell spectrum in which it grows to high titres, we let it grow in continuous cell lines such as CCL64, Vero, and L cells. We found that the enzymic activity in question was very different in these cells: in L cells being the lowest (specific activity 0.85) and relatively high in CCL64 and Vero cells (specific activities 7.6 and 14.6, respectively). The activity of cell-associated enzyme was not stimulated with 1 % Triton X-100. The activity of virion-associated CNPase was similar in VSV grown in CCL64 cells and CEC, although the cell-associated enzyme activity was much higher in CCL64 cells. The virion-associated enzyme activity was related to the cell-associated one in VSV grown in L cells (low) as well as in Vero cells (high). The relative enzyme activity associated with all examined cytopathic viruses was always lower as compared to that found in the host cells confirming the results obtained in CEC-grown viruses.

Retroviruses behave in a quite different manner. We have chosen the bovine leukaemia virus (BLV) and the avian myeloblastosis virus (AMV). The latter multiplies in myeloblasts, transformed mononuclear leukocytes. Because of the lack of necessary amount of noninfected control cells (leukocytes), the relative CNPase activity for AMV we calculated from the enzyme levels in the infected chick myeloblasts (the enzyme activity in chick myeloblasts was 1.8 times higher than in CEC and was not stimulated with Triton X-100). The purified AMV had quite unusual 3'-CNPase values. Intact virions showed activities similar to other viruses but in the presence of 1 % Triton X-100 the enzyme activity increased considerably (50–70 times); such high increase was found at AMV preparations coming from two different laboratories. Exclusively this virus revealed in the presence of 1 % Triton X-100 so extremly increased 3'-CNPase activity (15–30 times) in comparison to host cells. This result shows that 3'-CNPase accumulates inside of AMV virions.

BLV multiplied in foetal lamb kidney cells (FLK) clone GI/II derived from FLK cells persistently infected with BLV (Altaner *et al.*, 1985). BLV does not transform these cells. FLK-GI/II cells showed a low CNPase activity and, as expected, also the purified BLV-associated enzyme activity was low and, in addition, it could not be stimulated by 1 % Triton X-100. This was the only case when the detergent did not enhance the virion CNPase activity.

Finally, we aimed to determine the correlation between cell-associated 3'-CNPase and virus yield. We used VSV grown in L cells (low enzyme activity)

and in Vero cells (high enzyme activity). As shown in Fig.1, the virus grow better and quicker in Vero cells reaching 4 times higher yields than in L cells, similarly as described earlier (Paucker, 1970). The VSV yield in Vero cells was 400 PFU/cell and $1x10^8$ PFU/mg protein. The VSV yield in L cells was 90 PFU/cell and 3×10^7 PFU/mg protein. Similar differences in the VSV yields were obtained in CCL64 (high CNPase activity) versus CEC cells (low CNPase activities). VSV destroys Vero, CEC, and CCL64 cells when reaching optimum virus titre (by 24 hr post-inoculation the cells are destroyed). Only L cells behaved in a different way. By 24 hr p.i. their morphology was unchanged and

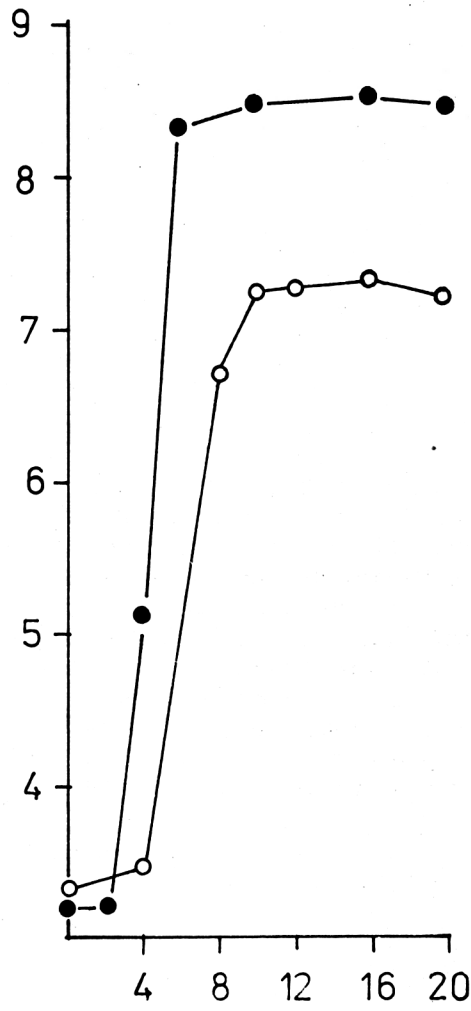

Fig. 1
Time course of VSV formation
VSV was propagated in Vero cells (●) and in L cells (○). Abscissa: hours of incubation; ordinate: plaque forming units (PFU) per ml.

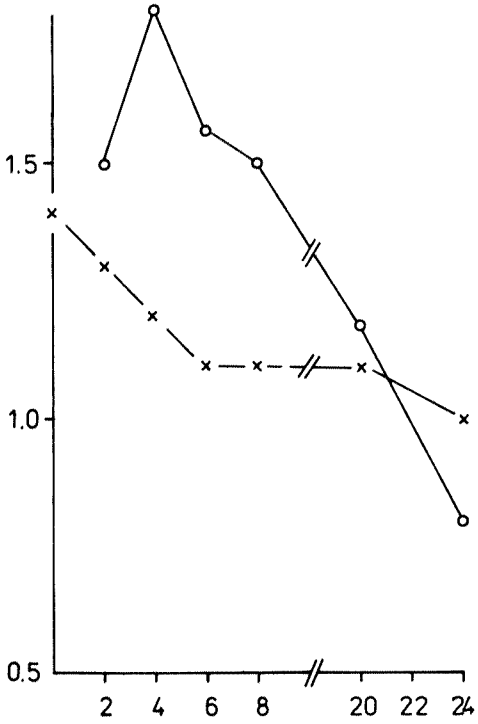

Fig. 2
Activity of 2', 3'-cyclic nucleotide 3'-phosphohydrolase in L cells infected with VSV
Abscissa: hours of incubation; ordinate 3'-CNPase activity of noninfected (×——×) and VSV infected (○——○) cells.

the cells remained well attached, but they were unable to divide even when anti-VSV serum had been added and later on (by 48 hr) these cells underwent cytopathic degeneration. Replication of VSV in different cells was followed by many investigations, but the reason for different host cells was not satisfactorily explained. It has been attributed to different metabolic conditions (Edelman and Wheelock, 1968; Nowakowski *et al.*, 1973).

In parallel experiments we followed the influence of virus replication on the activity of host-cell 3'-CNPase. In Vero cells (high enzyme activity) the enzyme activity was similar in infected and noninfected cells. Comparison of 3'-CNPase activity in VSV-infected and control L cells (low enzyme activity) is shown in Fig. 2. Noninfected L cells showed a slight decrease of 3'-CNPase activity within 24 hr. In infected cells, however, the enzyme activity increased by 35 % at 2–8 hr p.i., when the synthesis of virus components proceded.

We have shown that VSV synthesis in cells with high 3'-CNPase activity was considerably higher than the synthesis of this virus in cells with low activity of this enzyme. This result as well as the increase of 3'-CNPase activity during virus replication (L cells) reflect the possible role of this host-cell enzyme during virus replication.

References

Altaner, Č., Bán, J., Zajac, V., Rössler, H., Rosenthal, Z., Kettman, R., and Burny, A. (1985): Isolation and characterization of cell clones producing various amounts of bovine leukosis virus. *Folia biol.* **31,** 107–114.

Dreiling, Ch. E., Schilling, R. J., and Reith, R. C. (1981): 2', 3'-cyclic nucleotide 3'-phosphohydrolase in rat liver mitochondrial membranes. *Biochim. Biophys. Acta* **640,** 114–120.

Dulbecco, R., and Vogt, M. (1954): Plaque formation and isolation of pure lines with poliomyelitis viruses. *J. exp. Med.* **99,** 167–182.

Edelman, R., and Wheelock, E. R. (1968): Specific role for each human leukocyte type in viral infection. II. Phytohemagglutinin-treated lymphocytes as host cells for vesicular stomatitis virus. *J. Virol.* **2,** 440.

Lees, M. B., Sandler, S. W., and Eichberg, J. (1974): Effect of detergens on 2', 3'-cyclic nucleotide 3'-phosphohydrolase activity in myelin and erythrocyte ghosts. *Neurobiol.* **4,** 407–413.

Nowakowski, M., Feldman, J. D., Kano, S., and Bloom, B. R. (1973): The production of vesicular stomatitis virus by antigen- or mitogen-stimulated lymphocytes and continuous lymphoblastoid lines. *J. exp. Med.* **137,** 1042.

Paucker, K., Schechmeister, I. L., and Birch-Andersen, A. (1970): Studies on the multiplication of vesicular stomatitis virus with fluorescein and ferritin-conjugated antibodies. *Acta Pathol. Microbiol. Scand. (B)* **78,** 317.

Pristašová, S., Rosenbergová, M., and Holý, A. (1981): Decyclizing phosphodiesterase associated with plasma membranes of the chick chorioallantois. *Coll. Czechoslov. Chem. Commun.* **46,** 520–524.

Rosenbergová, M., Pristašová, S., and Prachař, J. (1981): Hydrolysis of ribonucleoside 2', 3'-cyclic phosphates by influenza and Newcastle disease viruses. *Acta virol.* **25,** 71–77.

Rosenbergová, M., Slávik, I., and Hána, L. (1981): Zonal density gradient electrophoresis of influenza A virus. *Acta virol.* **25,** 78–86.

Rosenbergová, M., and Pristašová, S. (1990): Localization of the 2', 3'-cyclic nucleotide 3'-phosphohydrolase in the virion of NDV. *Acta virol.* **34,** 508–516

Trapp, B. D., Bernier, L., Andrews, S. B., and Colman, D. R. (1988): Cellular and subcellular distribution of 2', 3'-cyclic nucleotide 3'-phosphodiesterase and its mRNA in the rat central nervous system. *Neurochem.* **51,** 859–868.