Acta virol. 24: 149—152, 1980

SINDBIS VIRUS RECEPTOR PROTEIN OF BHK CELLS

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Received July 10, 1979; revised September 18. 1979

Summary. — Exposure of cultured BHK 21 cells to very low concentrations of non-ionic detergent Nonidet P40 resulted in the elution of cellular proteins located on the outer surface of the plasma membrane. One of these proteins, partially purified by affinity ultrafiltration and cosedimentation with Sindbis virions, seems to be the "receptor molecule" of Sindbis virus.

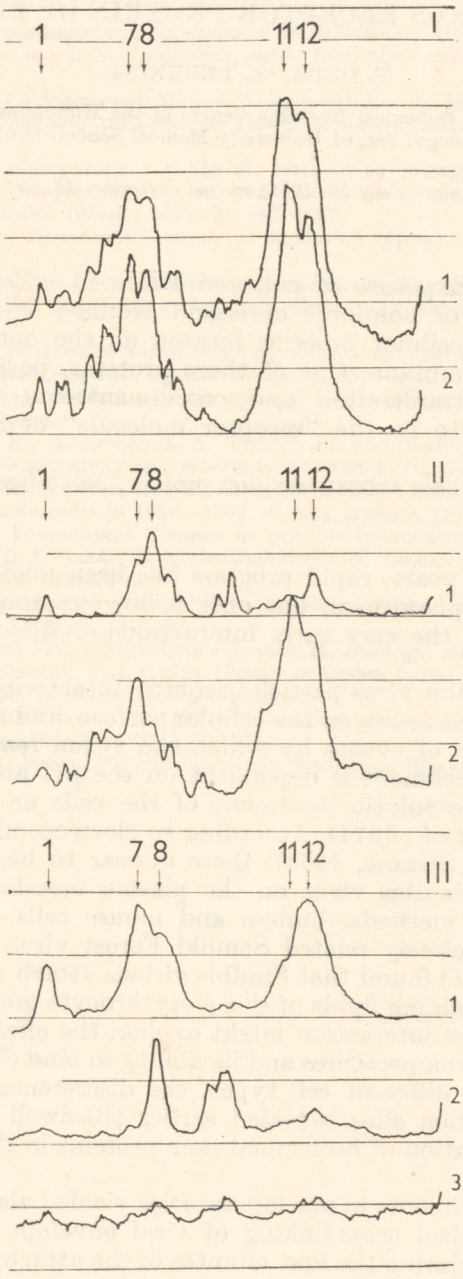
Key words: *Sindbis virus*; *receptor protein*; *solubilization*

Over the last few years, rapid progress has been made in understanding the replication of alphaviruses, but only a few experimental data became available concerning the very early interactions of these viruses with their host cells.

Adsorption of Sindbis virus particles requires intact virus and the presence of specific receptor molecules on the cellular surface, and does not necessarily lead to the sequence of events by which the virion reaches the site of its replication. The attachment is dependent on the pH and ionic strength of the medium and proteolytic treatment of the cells or the virions (Waite *et al.*, 1970; Pierce *et al.*, 1974). According to electron microscope investigations (Birdwell and Strauss, 1974), there appear to be approximately 10^5 reception sites for Sindbis virus on the plasma membrane of BHK cells. As found by other methods, human and mouse cells are able to adsorb 2×10^5 particles of closely related Semliki Forest virus (Fries *et al.*, 1978).

Mooney *et al.* (1975) found that Sindbis virions attach to artificial protein-free liposomes containing lipids of sheep erythrocyte membranes. While the (glyco) lipid — virion interaction might explain the effective attachment of Sindbis virus at low temperatures and its ability to bind (losely) to the plasma membrane of many different cell types, the discreteness and clustering of Sindbis virus reception sites revealed earlier (Birdwell and Strauss, 1974) suggest the participation of host membrane proteins in the process of (tight) binding.

Preliminary experiments in our laboratories yielded also some evidences in this direction. Chemical cross-linking of viral envelope proteins with host membrane proteins during the first minutes of the attachment period showed preferential participation of some host proteins in these complex molecules.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7 8
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Table 1. Effect of extracted proteins on Sindbis virus infectivity

Virus added PFU/ml	Number of plaques			
	Control	NP40 extract	XM-300 conc.	"receptor"
2×10^1	22	12	0	0
2×10^2	178	57	1	0
2×10^3	>200	243	27	2
2×10^4	>200	>200	>200	11

Virus was mixed with samples (adsorption period was 4 hr at 4 °C), the cells were washed and overlaid with agar. Plaques were counted after 40 hr. NP40 concentration was below 0.003% in the assayed preparation, "receptor" was freed of virus particles in a buffer of high ionic strength by centrifugation.

"NP40 extract", "XM-300 conc." and "receptor" samples contained amounts of receptor protein equivalent to the amount extracted from 10^8 BHK cells.

To study the molecular mechanisms underlaying the adsorption process, we attempted to purify the host protein(s) that recognizes (or is recognized by) the virus. An abstract has appeared (Duda and Berencsi, 1978).

We based our purification procedure on the observation that very low concentrations (0.01--0.03%) of non-ionic detergents (Triton X-100 or Nonidet P40) (NP40) (both polyoxyethylene--octylphenolethers) extract cellular proteins located on the outer surface of the plasma membrane (Pearlstein and Seaver, 1976).

BHK 21 cells were labelled on the surface by the lactoperoxidase-^{125}I method (Philips anr Morrison, 1970) and extracted with an ice could solution of 0.01% NP40 in phosphate buffered saline (PBS) for 30 min under occasional shaking of the Petri dishes. The NP40 extract was clarified by centrifugation to remove detached cells and debris. The extracted proteins were concentrated and NP40 was removed by ultrafiltration. This extract was mixed with Sindbis virus particles (2-5 volumes, 10^{10} virions/ml). The virus sample had been previously purified on a sucrose gradient, dialyzed and concentrated in an Amicon ultrafiltration unit. The mixture of viral particles and iodine-labelled cell surface proteins were again ultrafiltered on an Amicon type XM-300 filter.

Fig. 1.

Purification of Sindbis virus receptor protein of BHK cells

BHK cells were iodinated by lactoperoxidase and the labelled surface proteins were extracted by 0.01% NP40. Extracted proteins from approx. 10^8 cells were mixed with 10^{10} virus particles and the mixture was concentrated using an Amicon type XM-300 filter. Finally, the virus particles and bound proteins were pelleted through a 10% sucrose cushion at 280 000 × g for 3 hr; 400 mM NaCl in the buffer solution diminished binding of the protein to virions. Samples of radioactive proteins were analysed on sodium dodecyl sulphate polyacrylamide gel slabs; autoradiograms were scanned with a Gilford 250 spectrophotometer at 650 nm.

I — Lactoperoxidase-labelled surface proteins of BHK cells; cells were lysed with 1% NP40 after labelling (1) or labelled proteins were extracted with 0.01% NP40 (2).

II — Distribution of extracted surface proteins; pattern of radioactive proteins after ultrafiltration in the concentrated virus suspension (1) and flowthrough (2).

III — Proteins present in the supernatant after ultracentrifugation (1) and proteins bound to virus particles in the presence (3) or absence (2) of 400 mM NaCl.

Numbers refer to major protein bands, No. 8 with an approx. molecular weight of 45 000 is the "receptor protein".

As a next step the concentrated sample was further purified on a velocity gradient: virus particles and strongly bound proteins were pelleted through a sucrose cushion. Since adsorption of radiolabelled virus particles to host cells shows a sharp optimum at an ionic strength of 0.16 to 0.20 (Pierce *et al.*, 1974), parallel gradients with different ionic strengths were run.

Fig. 1 shows the purification steps and ionic strength-dependent binding of the extracted proteins.

Our purification procedure should be considered as a partial one, because proteins and glycopeptides that cannot be labelled by the lactoperoxidase method in situ are also extracted by NP40. Some of these proteins were present in our preparations, although not detected by autoradiography.

The purified "receptor" protein exhibits several properties of a hypothetical reception molecule: it is located on the outer surface of host cell plasma membrane, strongly and reversibly binds to virus particles, and inhibits virus attachment to host cells reducing virus infectivity (Table 1).

Although the yield of our procedure is unsatisfactory, it seems likely that gentle solubilization of the Sindbis virus receptor protein does not diminish its activity and improved methods for larger scale purification can start with the solubilization of plasma membranes.

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