J. gen. Virol. (1975), **29**, 133–136
Printed in Great Britain

Some Physicochemical Properties of Pike Fry Rhabdovirus RNA

(*Accepted* 4 *July* 1975)

SUMMARY

Pike fry rhabdovirus (PFR) RNA has been characterized as a single-stranded non-segmented molecule of about 4×10^6 daltons. In sucrose gradients in 0·1 M-NaCl it has a sedimentation coefficient of 40 to 45 S, somewhat lower than that of Semliki Forest virus RNA. The sedimentation velocity of the RNA is strongly influenced by the salt concentration and divalent cations. It shows a buoyant density of 1·65 g/ml in caesium sulphate. The base composition of the RNA is 21·6 % G, 25·1 % A, 22·4 % C and 30·9 % U. It is thus comparable to the RNAs of other rhabdoviruses.

Physicochemical properties of the RNAs of fish rhabdoviruses had not been reported until 1974 when McCain, Fryer & Pilcher (1974) presented some data on infectious haematopoietic necrosis virus (IHN). Quite recently, a detailed study about PFR structural constituents and the products of the virion-associated polymerase reaction has been published (Roy *et al.* 1975). We here confirm some of these findings and present additional data on the RNA of PFR.

Fathead minnow (FHM) cells and PFR (a kind gift from Dr P. de Kinkelin, INRA, Laboratoire d'Ichtyopathologie, Thiverval-Grignon, France) were grown as described previously (de Kinkelin, Le Berre & Lenoir, 1974). Virus was harvested 24 h after infection, precipitated with polyethyleneglycol and purified in linear 20 to 50 % (w/w) sucrose gradients in TES buffer (0·1 M-NaCl, 1 mM-EDTA, 10 mM-tris-HCl, pH 7·4). Virus was labelled either by adding 20 μCi/ml of [5-^{3}H]-uridine or 100 μCi/ml of carrier free [^{32}P]-orthophosphate (in phosphate free medium) to the cultures, 1 h after infection. Labelled RNA was extracted from purified virus by the phenol-SDS method. To avoid hydrolysis of the RNA with ribonuclease the extraction buffer was supplemented with 20 μg/ml of polyvinylsulphate. After precipitation with ethanol, the RNA was dissolved in water which had been treated with diethylpyrocarbonate (0·1 %, removed by autoclaving).

Sedimentation behaviour of the RNA under different salt conditions was studied in isokinetic gradients. Parameters for the construction of these gradients (10 % top concentration) have been taken from Van der Zeijst & Bloemers (1975), assuming a density of virus RNA of 2·00 g/ml in sucrose. Extracted RNA or disintegrated virus (2 % SDS, 5 min at 37 °C) was mixed with marker RNAs (disintegrated eukaryotic ribosomes at 10 μg RNA per gradient) and applied to the gradients.

Gradients containing 0·1 M-NaCl (TES buffer) revealed an RNA of 40 to 45 S, as calculated using the formula from Van der Zeijst & Bloemers (1975) or using the position of the ribosomal RNAs (Fig. 1 *a*). Lowering the salt concentration to 1 mM decreased the sedimentation coefficient to about 20 S (Fig. 1 *b*). The effect of divalent cations was studied in buffers with the same ionic strength as TES buffer. One mM-$MgSO_4$ increased the sedimentation velocity to 51 S (Fig. 1 *c*); RNA in gradients containing 1 mM-$CaCl_2$ showed identical sedimentation velocities (Fig. 1 *d*). The effect of different salt concentrations and divalent cations indicates the single-stranded character of PFR-RNA.

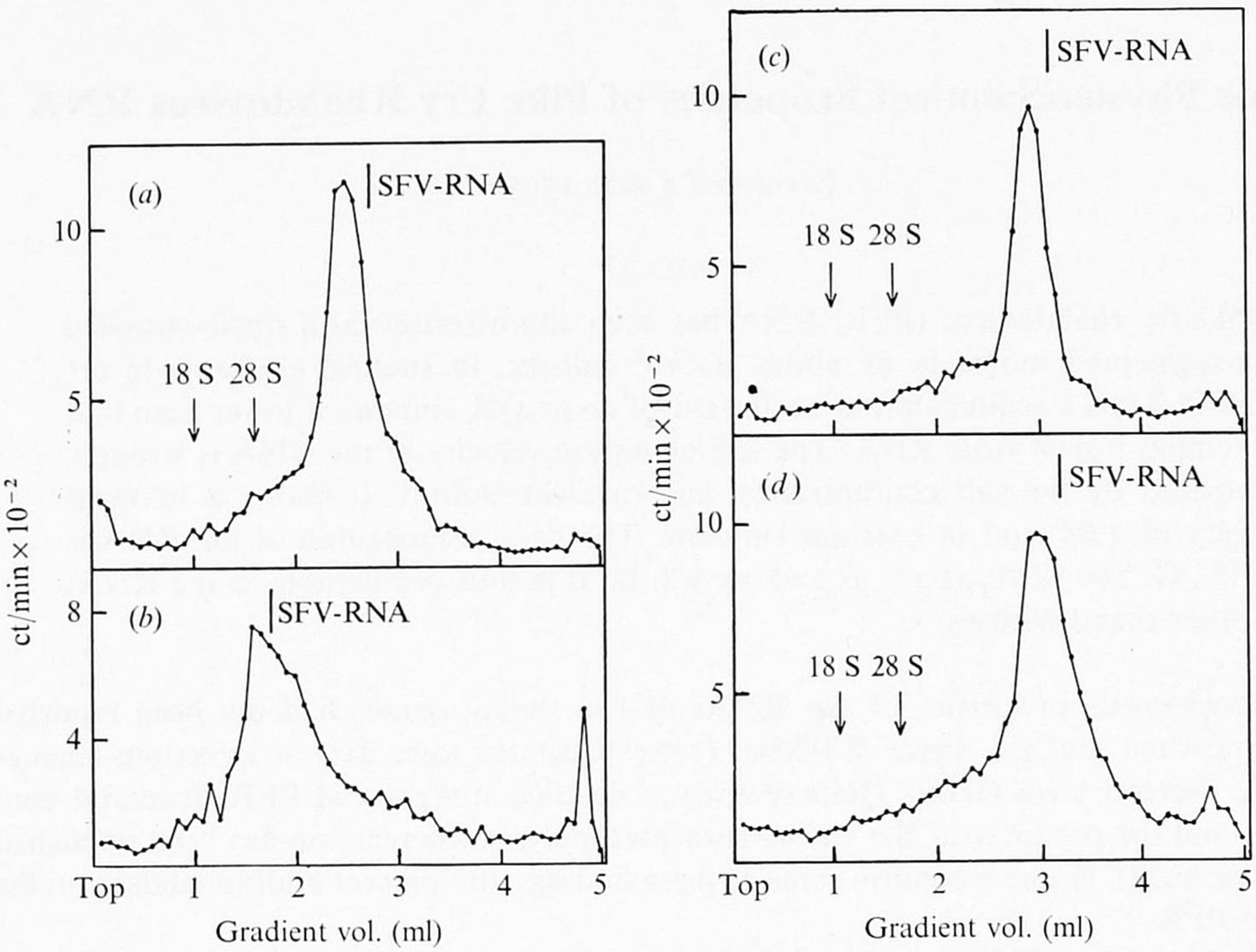

Fig. 1. Sedimentation behaviour of PFR-RNA in isokinetic gradients under different salt conditions. Gradients were centrifuged for 1·5 h at 50000 rev/min and 5 °C in a SW 50.1 rotor. (*a*) TES buffer: 0·1 M-NaCl, 1 mM-EDTA, 10 mM-tris-HCl, pH 7·4; (*b*) low salt buffer: 1 mM-NaCl, 1 mM-EDTA, 10 mM-tris-HCl, pH 7·4; (*c*) 1 mM-$MgSO_4$, 0·1 M-NaCl, 10 mM-tris-HCl, pH 7·4; (*d*) 1 mM-$CaCl_2$, 0·1 M-NaCl, 10 mM-tris-HCl, pH 7·4. Arrows indicate the positions of 18 and 28S marker RNAs. Bars at the top of graphs show the position of SFV-RNA.

For comparative purposes Semliki Forest virus (SFV) RNA was centrifuged in the same or duplicate gradients. The sedimentation coefficients of SFV-RNA were consistently slightly higher than those of PFR-RNA, irrespective of the composition of the gradients (Fig. 1 *a* to *d*) indicating a slightly smaller RNA in PFR virions.

Isokinetic sucrose gradients (10 % top concentration) containing 3 % (v/v) formaldehyde in phosphate-SDS buffer (Simmons & Strauss, 1972) were used to establish the colinearity of the viral genome. Before application to the gradients, RNA and marker RNA in TES buffer containing 3 % formaldehyde and 0·2 % SDS were incubated for 5 to 10 min at 37 °C. After centrifuging for 3 h at 50000 rev/min and 5 °C in a SW 50L rotor, only one peak of radioactivity was found sedimenting about 3 times slower than in gradients without formaldehyde. However, the non-segmented character of the RNA molecule must be considered with some reserve because denaturation may be incomplete at this temperature (Boedtker, 1968). Electrophoresis of the RNA was performed in composite polyacrylamide-agarose gels (Peacock & Dingman, 1968). Gels containing 1·85 % (w/v) acrylamide, 0·09 % bis-acrylamide and 0·5 % agarose in tris-acetate buffer pH 7·2 were soaked for 48 h in electrode buffer (40 mM-tris-acetate pH 7·2 with 0·2 % SDS (Bishop, Claybrook & Spiegelman, 1967) and pre-run for 1 h. Electrophoresis was carried out for 2 h at room temperature at 10 V/cm. Estimations of the mol. wt. using the migration distances of

Table 1. *Base composition of selected Rabdoviridae*

	%G	% A	% C	% U	References*
Fish rhabdoviruses					
PFR	21·6 ± 1·7	25·1 ± 1·0	22·4 ± 1·4	30·9 ± 1·7	this study
	20·2	24·9	22·5	32·4	Roy *et al.* 1975
IHN	24·2 ± 1·6	22·5 ± 1·2	25·4 ± 0·9	27·7 ± 0·5	McCain *et al.* 1974
Warm blooded animal viruses					
VSV Indiana	20·0 ± 1·8	27·4 ± 1·4	21·9 ± 1·1	30·8 ± 1·9	
Rabies	21·1 ± 0·7	26·4 ± 0·1	23·1 ± 0·9	29·4 ± 0·1	
Plant viruses					
Potato yellow Dwarf virus	21·4 ± 1·5	28·9 ± 0·1	20·5 ± 1·5	28·9 ± 1·2	

* Values for non-fish rhabdoviruses have been taken from Knudson (1973).

marker RNAs ranged betweeen 3·6 and $4{\cdot}2 \times 10^6$. In most experiments a mol. wt. of about $4{\cdot}0 \times 10^6$ was found, somewhat higher than the value found by Roy *et al.* (1975), possibly caused by the inaccuracy in extrapolation from the ribosomal RNAs.

The buoyant density of the RNA was determined in pre-formed Cs_2SO_4 gradients (32 to 70 %, w/w, in TES buffer). After centrifuging at 42000 rev/min and 25 °C, the refractive index of every second fraction was measured for the calculation of the density (Hearst & Vinograd, 1961). Centrifuging for 16 h resulted in a sharp peak at a density of 1·65 g/ml which did not alter after prolonged centrifuging (24 h). This value is in better agreement with those from non-fish rhabdoviruses than with the value for IHN virus RNA (1·59) reported by McCain *et al.* (1974).

Alkaline hydrolysates of [^{32}P]-labelled RNA were electrophoresed on Whatman 3 MM filter paper soaked in citric acid-citrate buffer, pH 3·5 (Davidson & Smellie, 1952) at 3000 V for 90 min at 2 °C together with 25 μg of 2′ and 3′ monophosphates. Spots were located under U.V. light, cut out and counted. Identical migration of labelled monophosphates with markers was confirmed by autoradiography. Values obtained from several determinations are given in Table 1, together with base compositions of some other rhabdoviruses. As can be seen, the base composition of PFR is comparable to those of other animal rhabdoviruses.

We thank Miss J. A. A. M. van den Heiligenberg for preparation of the manuscript.

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(*Received* 7 *April* 1975)