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# Buoyant Density of Hirame Rhabdovirus (HRV) in Cesium Chloride and Sucrose

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Hirame rhabdovirus (HRV) was first isolated from moribound Japanease flounder in 1984 and the structural proteins have been characterized.[2,5] The buoyant density of a virion in sucrose or cesium chloride (CsCl) gradients is important both for the clasification of viruses and to provide a method for purification and concentration of the viral particles. The buoyant density of rhabdoviruses is 1.19 to 1.20 g/cm$^3$ in CsCl and 1.17 to 1.19 g/cm$^3$ in sucrose.[3] McCain *et al.*[4] reported that the density of fish rhabdovirus, infectious hematopoietic necrosis virus (IHNV), was about 1.16 g/cm$^3$ in sucrose.[4] In this study we report the density of hirame rhabdovirus was 1.18 g/cm$^3$ in CsCl and 1.16 g/cm$^3$ in sucrose.

HRV strain 8401-H and IHNV were used in this study. IHNV was kindly provided by Dr. B. J. Hill, Fish Disease Laboratory, Ministry of Agriculture, Fisheries and Food, U. K. Both HRV and IHNV were propagated using fathead minnow (FHM) cell line[1]. The cells were grown at 15°C in Eagle's minimum essential medium (MEM; Gibco) supplemented with 10% fetal bovine serum (FBS; Gibco), 100 I.U./m*l* penicillin (Sigma), and 100 $\mu$g/m*l* streptomycin (Sigma). FHM cells were inoculated with virus at a low multiplicity of infection (0.01 to 0.001) and incubated at 15°C. After cell lysis, the culture fluid was collected and clarified by centrifugation (4,000 *g*, 10 min). Virus in the supernatant was precipitated by adding 7.0% (W/V) polyethylene glycol 6,000 (PEG-6,000) and 2.3% (W/V) NaCl. After centrifugation (3,000 *g*, 30 min), the pellet was resuspended in 1/10 volume of STE buffer (0.02 M Tris-HCl, 0.1 M NaCl, 1 mM EDTA, pH 7.4) and the mixture was centrifuged (4,000 *g*, 10 min) to remove the PEG. Viral particles in the supernatant were collected by centrifugation (80,000 g, 90 min). The pellet was resuspended in STE buffer containing 22.18% (W/W) of CsCl. After centrifugation (175.000 *g*, 20 h), the viral band formed in centrifuge tube was collected and centrifuged (150,000 *g*, 1 h) again to remove CsCl. The pellet was resuspended in STE-buffer containing 22.18% (W/W) CsCl and centrifuged for 12 h at 50,000 rpm by using analytical ultracentrifuge roter (RAP-60, Hitachi). The location of viral band formed in the analytical centrifuge tube was detected by Schlieren method (U.V. 260 nm)[6] and the density of virion was calculated by using the following expressions:

$$re=((rb^2+rm^2)/2)^{1/2}$$

$$Ps=Pe+(dP/dr)_{re}\times(rs\text{-}re)$$
$$=Pe+W^2\times re\times B^{-1}\times(rs-re)$$

re; distance from a centrifuge shaft to original density of CsCl
rb; distance from a centrifuge shaft to cell bottom
rm; distance from a centrifuge shaft to meniscus
rs; distance from a centrifuge shaft to sample
Ps; density of sample
Pe; Original density of CsCl
$W^2$; Angular velocity
B; A coefficient of CsCl density gradient

The sedimentation equilibrium pattern of HRV virion is shown in Fig. 1. Values of rm, rb, and rs were 6.753 cm, 7.219 cm, and 7.062 cm respectively, giving re value of 6.990 cm. The density of HRV virion was calculated from following values, Ps; 1.172, B; $2.110\times10^6$, $W^2$; $2.742\times10^7$. It was determined that the density of HRV virion in CsCl was 1.180 cm$^3$. In the case of IHNV, a reference isolate, the density was determined to be 1.171 g/cm$^3$. The density of HRV virion was a

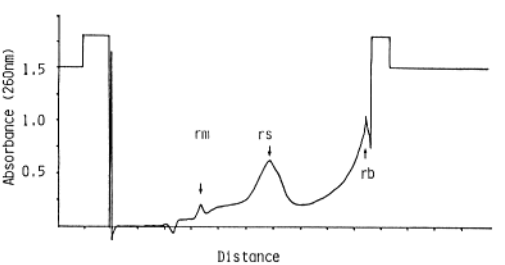


**Fig. 1.** Sedimentation equilibrium pattern of HRV virion at 260 nm in cesium chrolide after centrifugation at 50,000 rpm for 12 h. rm; meniscus, rs; sample, rb; cell bottom

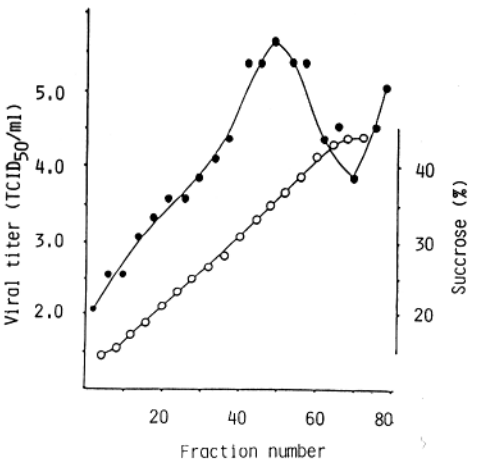


**Fig. 2.** Sedimentation equilibrium pattern of HRV virion in 8%–43% sucrose gradient after centrifugation at 80,000 g for 20 h. ●—●: viral infectivity, ○—○: concentration of sucrose.

little higher than that of IHNV, and those values were smaller than the range of density values for rhabdoviruses reported by Matthews[3].

The density of HRV virion in sucrose was measured by following method. Continuous sucrose gradients of 15–50% (W/V) were prepared in STE buffer and 0.3 m*l* of HRV (6.8 $TCID_{50}$/m*l*) was overlaid. After centrifugation at 80,000 *g* for 20 h, 50 $\mu l$ fractions were collected from top of the tube and aliquots were removed from each fraction for infectivity assays and sucrose density determinations. Infectivity was assayed by the end point dilution procedure ($TCID_{50}$) and sucrose density was determined from refractive index measurements. The sucrose gradient was linear and ranged from 8% to 43%. The highest infectivity titer of HRV was detected in fraction number 49 where the sucrose concentration was determined 35.5%. The density of this fraction was 1.160 g/cm$^3$. This was the same value for IHNV reported McCaln *et al.*,[4] but smaller than the range of values for rhabdovirus reported by Matthews[3].

## References

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