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Heterogeneity in the Hemagglutinating Agent of Japanese B Encephalitis Virus

The heterogeneity in the hemagglutinating agent of Japanese B Encephalitis Virus (JBEV) was found by the density gradient centrifugation technique.

The virus used was the Nakayama strain of JBEV grown in adult mouse brains. Infected brains were homogenized with 9 volumes of 0.14 M NaCl in 0.05 M Tris buffer, pH 8, (TBS) and spun down at 8,500 $\times g$ for 15 min.. The supernatant was treated with protamine sulfate (at a final concentration of 0.5 mg/ml) at 4°C for 60 min. and freed from precipitates by centrifugation (8,500 $\times g$, 15 min.). Partially purified virus was prepared from this supernatant by 2 cycles of differential centrifugation (90,000 $\times g$, 90 min. and 2,000 $\times g$, 15 min.).

Sucrose density gradient centrifugation was performed in a RPS 40 rotor of a Hitachi model 40 P centrifuge at 39,000 rpm for 4 hours, in a 20-49 per cent sucrose gradient in TBS. After the run, samples were divided into 10 fractions from the top of centrifuge tubes.

CsCl equilibrium density gradient centrifugation was performed following the method of Meselson *et al.*[1] in a SW 39 rotor of a Spinco model E centrifuge at 35,700 rpm for 40 hours. Samples were collected by droplet fractionation from the bottom of the tube. Density was determined refractometrically using the formula of Ifft *et al.*[2]

Hemagglutinating activity and hemagglutination inhibiting activity were estimated by Clarke and Casals' method.[3] Infectivity was titrated in four week old mice by intracerbral inoculation and titers were experssed as LD_{50}.

Sucrose gradient sedimentation of JBEV (both the protamine treated supernatant and partially purified virus) resulted in the separation of hemagglutinating activity into two components; rapid-sedimenting hemagglutinin (Hr) and slow-sedimenting hemagglutinin (Hs). Isolation of two peaks and recentrifugation showed that each formed a single peak at the position where it had originally been observed. Infectivity was also separated into two components. The peak of rapid-sedimenting infectivity was observed approximately in the same position as the Hr peak, but slow-sedimenting infectivity had its peak in a fraction just above the Hs peak (in the case of the protamine treated supernatant). When the protamine treated supernatant of normal mouse brain extract was centrifuged in a sucrose density gradient column under the same conditions as were used for the virus materials, a distribution of hemagglutination inhibiting activity was observed having its peak in a fraction just above the peak of the slow-sedimenting infectivity. So that deviation of the Hs peak from the peak of slow-sedimenting infectivity might be explained by a masking effect of some normal inhibitors for hemagglutinin.

Two peaks of hemagglutinin were also present in equilibrium density gradient columns of CsCl. When Hr and Hs isolated by sucrose gradient sedimentation were rebanded in CsCl, each formed a single peak at the position corresponding to the two hemagglutinin peaks of the original sample. The buoyant density of Hr is about 0.08 g/cc more than that of Hs (Table 1).

Table 1. Buoyant Density in CsCl Equilibrium Density Gradient Centrifugation

Initial density	Buoyant density of	
	Hs	Hr
1.24	1.241	1.317
1.25	1.243	1.325
1.26	1.247	1.318
average	1.244	1.326

Densities are expressed in g/cm^3 at 4°C

The hemagglutinating activities of Hr and Hs are inhibited by anti-Nakayama guinea pig serum to the same extent as the original material. The optimum pH for hemagglutination of Hr is about 0.2 pH unit less than that of Hs.

Separation of Hr and Hs can also be observed in different strains of JBEV grown in MK2 cells (K. Fujinaga, personal communication), so that it appears to be a rather general phenomenon in JBEV. The differences in density and optimal pH for hemagglutination suggest some differences in the chemical compositions of Hr and Hs, especially in their surface structures.

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