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Isopycnic Banding of Strains of Radish Mosaic Virus in Rubidium Bromide Solutions

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SUMMARY

Strains KV and HZ of radish mosaic virus when centrifuged to equilibrium in caesium chloride gave four bands, although they have only two nucleoprotein components. However, when centrifuged to equilibrium in rubidium bromide, HZ gave only two major bands and KV gave these and several extra small bands, presumably because this strain contains aggregates of 12 particles derived from all 3 components and therefore having different densities from the nucleoprotein components.

When centrifuged to equilibrium in CsCl solution, the bottom component of cowpea mosaic virus gives two bands. The relative amount of virus in the two bands differs from experiment to experiment and depends on the pH of the medium (Bruening, 1969; Van Kammen & Van Griensven, 1970; Wood, 1972). It was suggested that the extra band contained denser particles with bound caesium.

We have centrifuged two strains of radish mosaic virus, which is serologically related to cowpea mosaic virus, in CsCl solutions. KV is an English strain that forms aggregates of 12 particles and HZ is a strain obtained from Yugoslavia (Štefanac & Mamula, 1971) which does not form aggregates but, serologically, is very similar to KV (Kassanis, White & Woods, 1973). When centrifuged KV and HZ each gave 4 bands within the density range that included the middle and bottom components (Fig. 1), i.e. one more band than was given by the middle and bottom components of cowpea mosaic virus. Although the two strains each gave four bands, the proportion of material in the different bands differed between the strains. The presence of aggregates of 12 particles in KV preparations did not result in any extra bands, although infectivity tests suggested that the aggregates consisted of mixtures of particles from the middle and bottom components (Kassanis *et al.* 1973) and should therefore have densities between those of the middle and bottom components.

In attempts to prevent the formation of extra bands containing virus particles with bound caesium, we tried centrifuging KV and HZ in RbBr solutions. The virus was centrifuged in a Beckman model E analytical centrifuge for 18 h at 24 °C and 44700 rev/min in approximately 60% (w/v) RbBr solutions buffered to pH 7 with 0·066 M-phosphate. KV and HZ each gave two major bands corresponding to the middle and bottom components but between the two major bands of KV there were at least five small bands (Fig. 2). To find whether these small bands were given by the virus aggregates, we centrifuged middle and bottom components in a sucrose gradient and removed samples of aggregated and unaggregated virus (the methods were the same as in Kassanis *et al.* 1973). Each sample was then centrifuged to equilibrium in RbBr, and while the unaggregated virus, like HZ, produced only two bands, corresponding to the middle and bottom components (Fig. 3), the aggregates produced at least 15 small bands (Fig. 4). The bands absorbed u.v. light as shown in Fig. 5. If aggregates were composed only of middle and bottom component particles, there should have been no more than

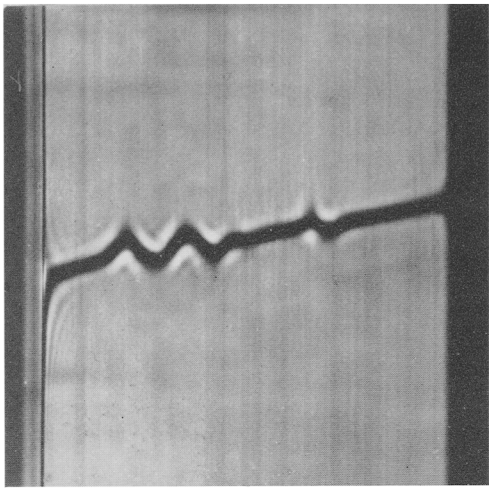

Fig. 1

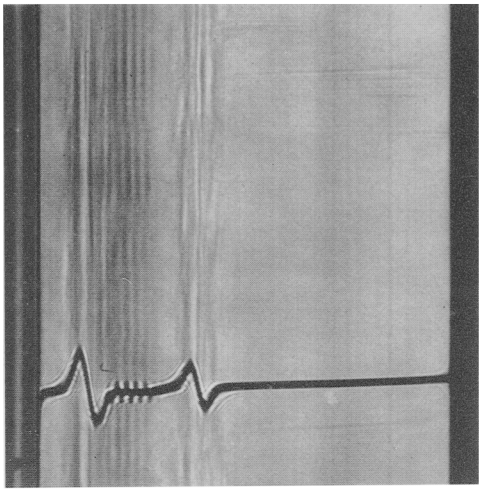

Fig. 2

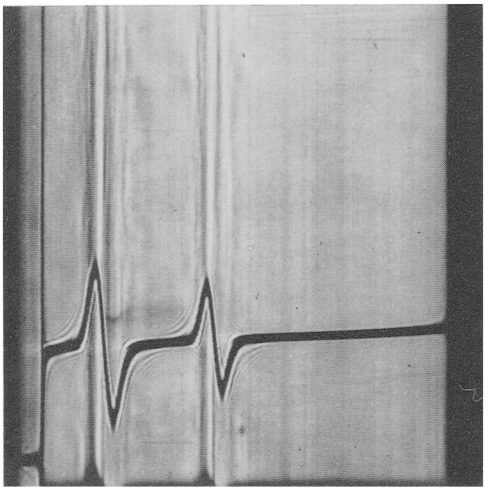

Fig. 3

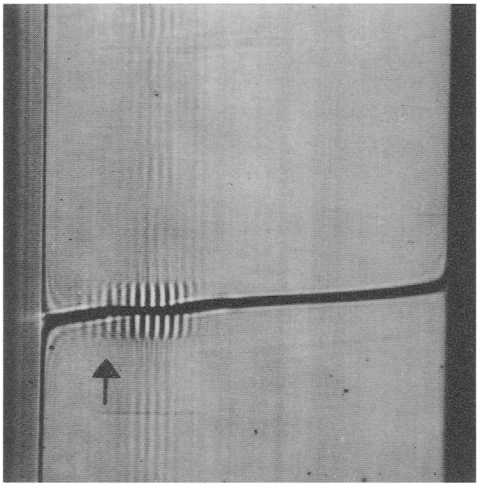

Fig. 4

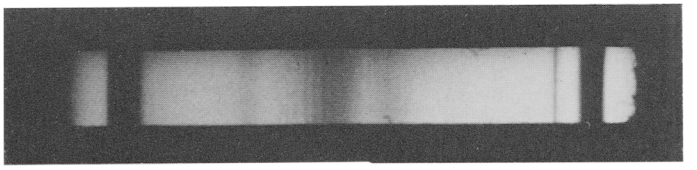

Fig. 5

Fig. 1. Isopycnic sedimentation of HZ at 0·25 mg/ml in CsCl (starting density 1·465 g/cm³).
Fig. 2. Isopycnic sedimentation of KV at 0·7 mg/ml in RbBr (starting density 1·471 g/cm³).
Fig. 3. Isopycnic sedimentation of unaggregated KV in RbBr (starting density 1·460 g/cm³).
Fig. 4. Isopycnic sedimentation of KV aggregates in RbBr (starting density 1·458 g/cm³).
Fig. 5. U.v. absorbing bands after isopycnic sedimentation of KV aggregates in RbBr.

13 bands in Fig. 4, which would represent all possible combinations of the two nucleoprotein components in the aggregates. Some bands were between the meniscus and the density level of the middle component band (indicated by an arrow in Fig. 4). Thus some aggregates are less dense than the middle component, and probably contain particles from the top component.

Centrifuging to equilibrium in RbBr gave a more accurate representation of the virus components that exist in aqueous solution than using CsCl. Equilibrium sedimentation of KV also detected aggregates which consist of particles of all three components. Moreover, after centrifuging to equilibrium in RbBr in a fixed partition cell, it was possible to separate the bottom component and show it to be infective after it was mixed with the middle component. For these reasons we think that RbBr is more useful for equilibrium sedimentations, at least for these two viruses. The fact that RbBr gives better results than CsCl is likely to be a specific ion effect rather than an ionic strength effect, since the two salts have roughly the same molarity at the banding densities.

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