Influence of pH and Divalent Anions on the Buoyant Density of Maize Dwarf Mosaic Virus in CsCl

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SUMMARY

The buoyant density of particles of maize dwarf mosaic virus (MDMV) in CsCl was influenced by pH and divalent anion concentration. Particle density increased with increasing pH at low buffer molarity; it decreased with increasing divalent anion concentration. The density shifts induced by divalent anions and acid pH were reversible, but those occurring at alkaline pH were irreversible. Of the strains examined, all behaved identically except 13B (MDMV-B), which was denser under all conditions and so could be physically separated from MDMV-A.

Wood (1971) observed that the buoyant density of cowpea mosaic virus components in CsCl depended on pH and Matthews (1974) reported similar pH-dependent density changes of turnip yellow mosaic virus. In other instances, buoyant density variation among strains of viruses have been described (Siegel & Hudson, 1959; Kellenberger, Zichichi & Weigle, 1961; Roizman & Roane, 1961; Hertel, Marchi & Muller, 1962; Lark, 1962; Goodheart, 1965; Magdoff-Fairchild, 1967).

Purification studies on MDMV revealed that changes in buffer concentration altered its buoyant density in CsCl (Gordon & Gingery, 1973). The influence of gradient composition and pH on the buoyant density of MDMV and attempts to differentiate strains of the virus by buoyant density are reported here.

The effect of buffer concentration on the buoyant density of MDMV was first observed with potassium phosphate (Gordon & Gingery, 1973). The relationship between potassium phosphate concentration and buoyant density of several MDMV strains is shown in Fig. 1(a). MDMV-A, -D, and -E (Louie & Knoke, 1970) were not significantly different. However, 13B (Gordon & Williams, 1970), a virus similar to MDMV-B (MacKenzie, Wernham & Ford, 1966), banded at a higher density at all concentrations of phosphate. In all cases, buoyant density decreased with increase in phosphate concentration.

Because potassium phosphate contributed to solution density, the amount of CsCl at the virus banding position decreased as the phosphate concentration increased. The CsCl concentration at the virus peak was found to range from about 2·3 M in 0·01 M-phosphate to about 0·7 M in 1·5 M-phosphate. To determine whether it was CsCl or potassium phosphate that affected the density of MDMV, other potassium salts were used (Fig. 1b). The concentration of Cs^+, K^+, Cl^-, or NO_3^- was without effect since no change in buoyant density was observed in the KNO_3 or KCl series. Only divalent anion salts caused significant shifts in buoyant density and these were reversible. Virus from zones in a phosphate series dialysed against 0·01 M-potassium phosphate, pH 7·o, and re-run in CsCl containing 0·01 M-potassium phosphate, pH 7·o, had the same buoyant density.

The influence of pH on the buoyant density of MDMV-A depended on the phosphate concentration (Fig. 1c). At 0·01 and 0·1 M-phosphate, the buoyant density increased with increasing pH. At 1·0 and 1·5 M-phosphate, the buoyant density decreased with increasing pH (except 1·0 M-phosphate, pH 9·0). At 0·5 M-phosphate, the effect was mixed. Although the buoyant density was inversely related to phosphate concentration at each pH, the rate
Fig. 1. (a) Influence of potassium phosphate concentration on the buoyant density of MDMV-A, -D, and -E, and 13B virus. About 50 μg virus, purified by the method of Gordon & Gingery (1973), was banded isopycnically in CsCl containing 0.01, 0.1, 0.5, 1.0, or 1.5 M-potassium phosphate, pH 7.0. Centrifugation was at 20 °C for 16 to 18 h at 43000 rev/min in the Beckman SW 50.1 rotor. (Increasing the centrifugation time to 72 h had no effect.) Gradient columns were fractionated in the ISCO Model 640 density gradient fractionator and scanned with the Model UA-2 ultraviolet analyser. Fractions (0.4 ml) were collected and their density determined by weighing a 50 μl sample in a pipette calibrated with water. Density of the gradient at the peak of virus concentration was considered to be the density of the virus. ★ ★ ★, 13B; ○ ○ ○, MDMV-D; □ □ □, MDMV-A; ● ● ●, MDMV-E. (b) Effect of salts on the buoyant density of MDMV-A. CsCl gradients were made in 0.01 M-potassium phosphate, pH 7.0, plus salt. Centrifugation and density determinations were carried out as in (a). ● ● ●, KNO₃; ○ ○ ○, KCl; □ □ □, potassium phosphate; ■ ■ ■, potassium tartrate; ★ ★ ★, K₂SO₄; ★ ★ ★, K₂SeO₄. (c) Influence of pH and potassium phosphate concentration on the buoyant density of MDMV-A. Density determinations in CsCl gradients containing 0.01, 0.1, 0.5, 1.0, and 1.5 M-potassium phosphate were made at pH 5.0, 6.0, 7.0, 8.0, and 9.0. The pH was adjusted just before centrifugation and after CsCl was added. Centrifugation and density determinations were done as in (a). Numbers in parentheses refer to phosphate concentration.
of density decrease varied; it was least at pH 5.0 and increased with pH. This probably reflected increases in concentration of the divalent anion, HPO$_4^{2-}$, with increasing pH.

The pH density shift was reversible when virus that had been centrifuged at pH 5.0, 6.0 and 7.0 in 0.01 M-potassium phosphate was recentrifuged at pH 7.0. The buoyant density of virus at pH 8.0 remained elevated when recentrifuged at pH 7.0, and virus at pH 9.0, 0.01 M-phosphate was unstable. For cowpea mosaic virus (Wood, 1971) and turnip yellow mosaic virus (Matthews, 1974) density changes resulting from alkaline gradients were irreversible. Under mild alkaline conditions, Verhagen & Bol (1972) and Matthews (1974) reported structural changes in alfalfa mosaic virus and turnip yellow mosaic virus, respectively. Unlike the other zones, the MDMV zone at pH 8.0 was skewed somewhat toward the dense end of the gradient and remained so after adjustment to pH 7.0 and recentrifugation. This suggested some sort of heterogeneity in the virus population induced by alkaline pH. One possible explanation is the partial loss of protein subunits from some virus particles at pH 8.0 and complete degradation at pH 9.0.

Virus used in these experiments was stored at 4 °C in 0.5 M-potassium phosphate, pH 7.0, with a small amount of chloroform. No change in density was observed during 2 months storage for any strain.

Density determinations were accurate to ±0.001 g/ml and reproducible to ±0.0015 g/ml based on 10 replicate determinations of MDMV-A in 0.5 M-phosphate, pH 7.0, done over the course of the experiments.

There appeared to be two general effects influencing virus density—a pH effect that increased virus density as pH was increased, and a divalent-anion effect that decreased virus density as the divalent-anion concentration was increased. The divalent-anion effect dominated over the pH effect at high concentrations of phosphate.

Increased buoyant density in CsCl with increasing pH has been observed for other macromolecules and may reflect a general phenomenon of alkaline titration in CsCl solutions (Vinograd, 1963). On the other hand, Rowlands, Sangar & Brown (1971) attributed increased density of picornaviruses with increasing pH to configurational changes in the virus particles caused by weakening of binding between RNA and protein. For MDMV, instability at alkaline pH supports the latter hypothesis.

The divalent-anion effect probably can be explained, in part, by differences in the solvation of MDMV. Hearst & Vinograd (1961) demonstrated that the buoyant density of bacteriophage T4 DNA depended on the extent of solvation, which was related to water activity; this, in turn, depended on the nature and concentration of solutes. However, water activity is affected by the concentration of both mono- and divalent anion salts, and as the MDMV density shifts were observed only with divalent anions, it seems unlikely that solvation alone can explain the divalent-anion effect. A preferential interaction of divalent anions with MDMV seems likely, and this may affect its reaction with caesium ions or its degree of hydration.

The results show that the density of MDMV depends on the method of determination. Both divalent anion concentration and pH affect the values. The results also show that the buoyant density of 1B is greater than that of the other MDMV strains suggesting structural differences between the viruses. This not only adds to previous demonstrations of differences between these viruses (MacKenzie et al. 1966; MacKenzie, 1967; Tu & Ford, 1969; Gordon & Williams, 1970; Snazelle, 1970), but also provides a convenient method for physically separating 1B from the other MDMV strains (except MDMV-B, presumably). Tosic & Ford (1974) observed a somewhat similar difference in buoyant density between MDMV-A and MDMV-B, although it was smaller than that reported here.
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REFERENCES


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