Relationships between the Multiplication of Chronic Bee-Paralysis Virus and its Associate Particle

By B. V. BALL,[1]* H. A. OVERTON,[2]† K. W. BUCK,[2] L. BAILEY[1]
AND J. N. PERRY[1]

[1] *Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ and* [2] *Department of Pure and Applied Biology, Imperial College of Science and Technology, London SW7 2AZ, U.K.*

SUMMARY

The amounts of chronic bee-paralysis virus associate (CPVA) produced in bees injected with purified chronic bee-paralysis virus (CPV) were negatively associated with the amounts, lengths of particles and maximum sedimentation coefficients of CPV. The ratio of the amounts of CPV/CPVA varied widely between individuals, but more CPVA was produced and CPV/CPVA ratios were smaller when CPVA was injected with the CPV preparations than when the latter were injected alone. The multiplication of the RNAs of CPV and of CPVA were also negatively correlated. The nature of the relationship between CPV and CPVA and the packaging arrangement of CPV RNA are discussed.

INTRODUCTION

Chronic bee-paralysis virus (CPV), which causes a trembling condition followed by death in the adult honey bee, *Apis mellifera*, has ellipsoidal particles falling into four size classes with modal lengths of about 30, 40, 55 and 65 nm and corresponding sedimentation coefficients ranging from 80S to about 130S (Bailey *et al.*, 1963; Bailey, 1976). In caesium chloride, the different components are of very similar buoyant density (1·33 g/ml) and they are serologically indistinguishable (Bailey, 1976; Bailey *et al.*, 1968). Particles of the longest (65 nm) size class had an LD_{50}/ml for adult bees up to 150 times greater than that of short (30 or 40 nm) particles at an equal A_{260} (Bailey, 1976).

Chronic bee-paralysis virus associate (CPVA) consists of 17 nm isometric particles which are serologically unrelated to CPV but are frequently found associated with it in the field (Bailey *et al.*, 1980). CPVA has never been isolated from healthy bees or in association with any other virus. When mixtures of CPV and CPVA are injected into queen bees (adults or pupae) or drone pupae, both viruses frequently multiply in large amounts, whereas they were extracted in similar amounts only occasionally from adult worker bees and in worker pupae only CPV usually multiplies. CPVA will not multiply when injected alone into any type of bee but, surprisingly, it occasionally multiplied in bees injected with highly purified preparations of CPV (Bailey *et al.*, 1980). This was explained when it was shown that the three RNA species of CPVA (RNAs 3a, 3b and 3c, all 0·35 × 10⁶ mol. wt.) could also be encapsidated very efficiently in CPV coat protein, along with the two RNA species of CPV (RNAs 1 and 2, 1·35 × 10⁶ and 0·9 × 10⁶ mol. wt. respectively) (Overton *et al.*, 1982). The properties of CPVA and CPV and their RNA components are summarized in Table 1.

This paper reports the effects of CPVA replication on the replication, size and RNA content of CPV particles.

† Present address: Department of Microbiology, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, U.K.

Table 1. *Properties of CPV and CPVA*

| | Virus particles | | RNA species* | |
| | | | | |
Virus	Dimensions or diameter (nm)	$s^0_{20,w}$ (S units)	Designation	Mol. wt. ($\times 10^{-6}$)
CPV	65 × 20	125–136	1	1·35
	55 × 20	110–124	2	0·9
	40 × 20	97–106	3a	0·35
	30 × 20	82	3b	0·35
			3c	0·35
CPVA	17	41	A (= 3a)	0·35
			B (= 3b)	0·35
			C (= 3c)	0·35

* Suggestions for packaging arrangements of the CPV RNA species in particles of different lengths are made in the Discussion section.

METHODS

Propagation of virus. The CPV was originally from colonies of bees with paralysis and produced a typical peak of three major components on a sucrose gradient (Bailey *et al.*, 1968). Later, CPV and CPVA propagated in queen pupae or adults and purified by one or more cycles of centrifugation on sucrose gradients were used.

The youngest pupal stages of the female and male reproductive caste (queens and drones respectively), or of workers, were removed from their cells, inoculated by injection and maintained in incubators at 35 °C (Bailey & Woods, 1974, 1977).

Adult bees were maintained in the laboratory and inoculated as described by Bailey & Woods (1974, 1977).

Purification and analysis of viruses. Individual queens were extracted in 8 ml 0·01 M-potassium phosphate buffer (PB) at pH 6·7, containing 0·02% sodium diethyldithiocarbamate with 1 ml diethyl ether. The mixture was then emulsified with 1 ml carbon tetrachloride, coarsely filtered, cleared at 3000 *g* for 30 min and then centrifuged at 100000 *g* for 2 h. After a further cycle of low and high speed centrifugation, extracts were layered on to sucrose density gradients (10 to 40% w/v) and centrifuged at 45000 *g* for 4·5 h at 15 °C. Fractions were collected from the top of the tube by displacement from beneath with 50% sucrose and assayed for A_{254} by means of an ISCO UA-2 analyser. The individual bands were dialysed against PB and then centrifuged at 100000 *g* for 2 h. The CPV from individual queen pupae was resuspended in at least 1 ml PB and sedimentation coefficients ($s_{20,w}$) were determined in a MSE Centriscan centrifuge. Occasionally, when there was insufficient CPV from a single pupa, extracts that had given similar gradient traces were pooled. Drone and worker pupae, in groups of five and 15 respectively, were extracted similarly.

The areas of the absorption curves of CPV and CPVA were measured from the sucrose gradient traces by a Quantimet 720 Image Analysing computer, or were cut from photocopies and weighed.

Purified preparations of CPV were negatively stained with neutral sodium phosphotungstate and examined and photographed in a Siemens Elmiskop electron microscope, internally calibrated with a ruled graticule (2160 lines/mm).

Analysis of RNA. Polyacrylamide gel electrophoresis (PAGE) of RNA, followed by staining and scanning of gels, were carried out as previously described (Overton *et al.*, 1982).

RESULTS

Effect of CPVA multiplication on CPV particle size and replication

Queen pupae were injected with highly diluted preparations of purified CPV, maintained for several days and virus was extracted as described in Methods. For individual queen pupae, measurements of the total amounts of CPV and CPVA present and of the maximum length and maximum S value (sedimentation coefficient) of the CPV particles were obtained.

The relationships of the area beneath the absorption curves of CPVA and CPV to the maximum S value of the CPV extracted from individual queen pupae that had been injected with purified CPV are given in Fig. 1 (*a*) and (*b*). Replication of CPVA had the effect of reducing the maximum length of CPV particles. This was shown by the highly significant ($P < 0·001$) negative correlation of the amount of CPVA with the maximum S values of CPV, together with the close correspondence of S values and particle lengths. Samples with low S values had particles with maximum lengths shorter than those with high S values.
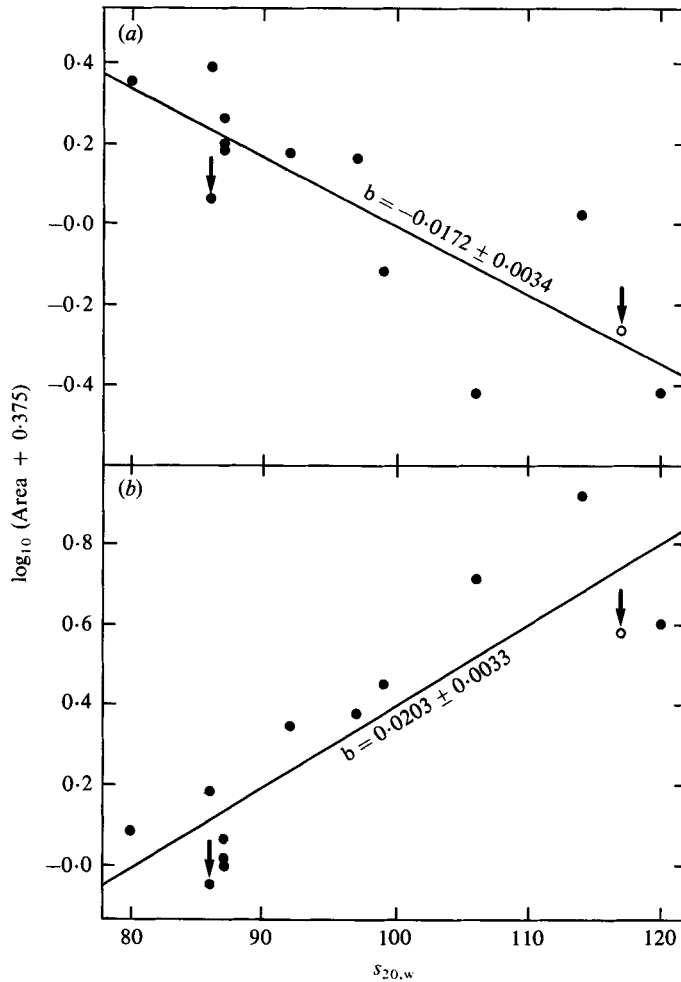
Fig. 1. Regression of logarithm of the area (cm^2) beneath the absorption curves of (*a*) CPVA and (*b*) CPV on the maximum sedimentation coefficient ($s_{20, w}$) of CPV, from individual queen pupae injected with apparently pure CPV. Arrows indicate values from two individuals injected with the same purified preparation of CPV; see also Fig. 2.

The lengths of particles of CPV from two individual queen pupae that had been injected with the same preparation of CPV, but that had produced different ratios of CPV to CPVA and very different S values of CPV (see Fig. 1*a*, *b*), had different frequency distributions (Fig. 2). There were more long particles of CPV when the relative amount of CPVA was low, and the three main modal lengths were about 42, 52, and 65 nm, which are characteristic of CPV preparations from field samples of paralysed worker bees (Bailey *et al.*, 1968). CPV from queen pupae that produced relatively more CPVA was composed almost entirely of short particles, with modal lengths of 36 and 42 nm, corresponding to the shorter modal lengths observed in preparations of CPV from field samples of paralysed worker bees (Bailey, 1976).

Replication of CPVA also had the effect of reducing the extent of CPV replication. The maximum S values of CPV showed a negative correlation with the amounts of CPVA, but a positive correlation with the amounts of CPV, indicating an inverse relationship between the amounts of CPVA and CPV. Evidence that this was due to interference by CPVA of CPV replication was obtained by injecting purified CPVA as a supplement to CPV into each of a further 10 individuals. Nine produced CPVA detectable as bands in sucrose gradients,
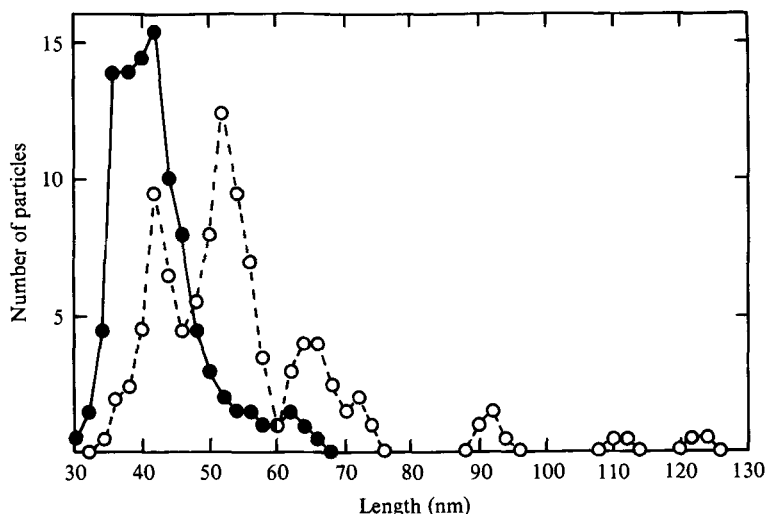
Fig. 2. Distribution of the lengths of particles of CPV from each of two queen pupae injected with the same purified preparation of CPV (see Fig. 1). Points are running means of two successive values. ●, CPV/CPVA = 0·7; ○, CPV/CPVA = 20.

significantly more (χ^2, $P < 0.02$) than the proportion of queens producing CPVA when injected with purified CPV only. Moreover, relatively much less CPV multiplied in the queens injected with CPV and CPVA than in those injected with CPV alone, ranging from almost undetectable amounts to less than one-half the amount of CPVA. A tenth queen pupa injected with CPV and CPVA survived.

The ratios of the amount of CPV/CPVA obtained after injection with either CPV alone or CPV and CPVA seemed not to conform to any common probability distribution and varied widely between individuals injected with the same preparation. Values of each observed ratio were therefore ranked, and the non-parametric Mann & Whitney (1947) U-test was used to assess whether the probability distributions of each treatment was the same. The null hypothesis of equality was rejected ($P < 0.002$) and ratios of CPV/CPVA obtained after injection with CPV and CPVA were smaller than those inoculated with CPV alone.

Similar results were obtained with worker and drone individuals but they produced much less CPVA than most queens, usually almost none, and showed less variation between the CPV/CPVA ratios and between the lengths of particles.

Effect of the multiplication of CPVA on CPV RNAs 1 and 2

The RNA of CPV samples from individual queen pupae was analysed by PAGE and the results from three representative individuals, together with the sucrose density gradient profiles of CPV and CPVA from these queens, are shown in Fig. 3. Since the samples for RNA analysis were prepared by resuspending the total CPV from one queen in a standard volume of buffer, the areas of the RNA peaks give direct measures of the amounts of encapsidated RNAs 1 and 2 in these bees (but not of the overall amount of RNA 3, since a variable amount of this has also been encapsidated in CPVA particles). It can be seen that the total amount of CPV RNAs 1 and 2 is greatly reduced as CPVA multiplication increases. This is consistent with the observations reported above that replication of CPVA interferes with the replication of CPV. The results in Fig. 3 also confirm the observations of Overton et al. (1982) that the proportion of CPVA RNA (RNAs 3a, b and c) encapsidated in CPV particles increases relative to RNAs 1 and 2 as CPVA replication increases.
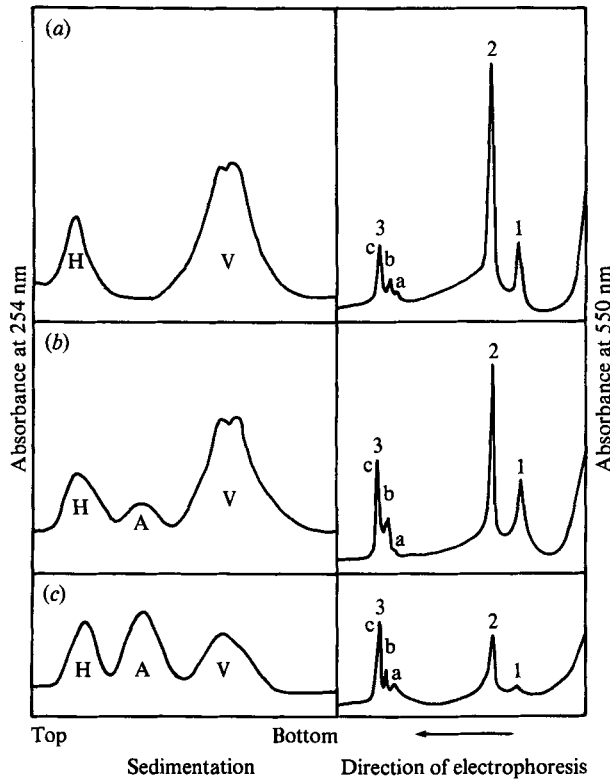
Fig. 3. Sucrose density gradient profiles of virus samples (left) and PAGE of RNA from their CPV fractions (right). Gels were stained with toluidine blue and scanned at 550 nm. Density gradients were scanned at 254 nm with an ISCO u.v. monitor. V, CPV; A, CPVA; H, host material. Samples (*a*), (*b*) and (*c*) were from three individual queen pupae injected with the same inoculum. The bands of CPV and CPVA were identified as described by Bailey *et al.* (1980).

Relationship between CPV RNA content and particle size

The negative correlations of CPVA replication both with CPV particle length and with replication of CPV RNAs 1 and 2, imply that the two latter properties are positively associated. This was also demonstrated more directly by examining the RNA content of CPV preparations and comparing these with the particle size distribution obtained from electron micrographs. Two contrasting examples are shown in Fig. 4. In sample (*a*), where there is a fairly even distribution of particles between the three largest size classes but few of the shortest particles, RNAs 3a, b and c form a minor part of the total RNA, whereas in sample (*b*), where the shortest particles are in the majority, the three smallest RNAs constitute more than half of the total.

DISCUSSION

CPVA RNA is encapsidated in a protein coat which is serologically unrelated to that of CPV and which is presumably encoded by one or more of RNAs 3a, b and c. However, replication of CPVA is absolutely dependent on CPV as a helper virus, probably to provide protein(s) required for RNA replication. Hence competition between CPV and CPVA for such proteins would be expected, as has been found with other helper virus-dependent systems, such as satellites (Kassanis, 1962; Kaper & Tousignant, 1977; Schneider, 1970; Murant *et al.*, 1973) and defective interfering particles (Huang & Baltimore, 1977). The results of infections when CPVA was added to a CPV inoculum show clearly that CPVA can interfere with the replication of CPV.

The production of CPVA when pupae were injected with highly purified preparations of CPV is presumably due to CPVA RNA (RNAs 3a, b and c) encapsidated by CPV. In such cases it
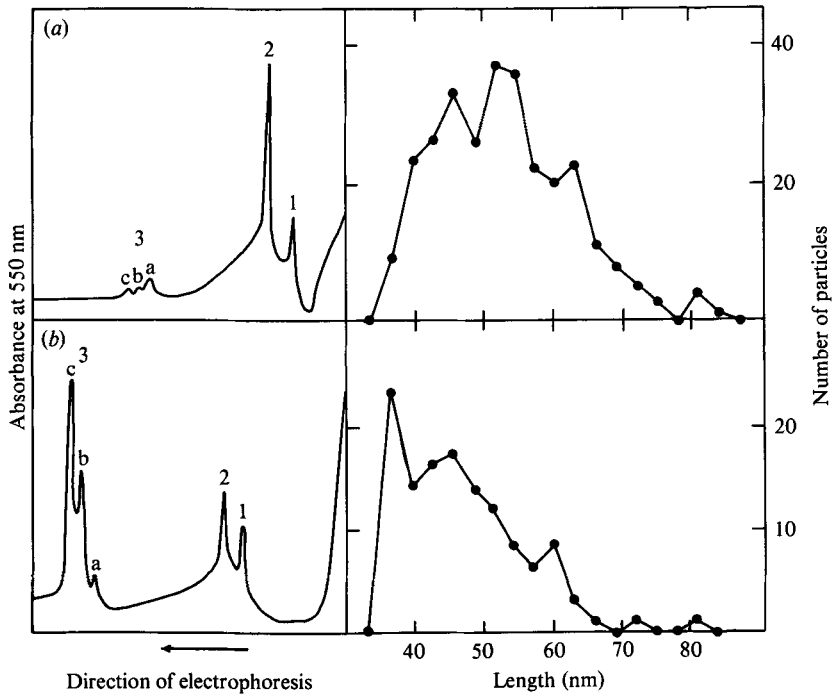
Fig. 4. PAGE of RNA (left) and distribution of the lengths of particles (right) from two CPV preparations. RNA was electrophoresed and analysed as described in the legend to Fig. 3.

would be expected that CPV/CPVA ratios would depend on the relative proportions of RNAs 1, 2 and 3 (a, b and c) in the CPV inoculum. However, the variability in the relative amounts of CPV and CPVA extractable from individual queen pupae inoculated with the same amount of the same preparation of CPV (Fig. 1 and 3) suggest that their multiplication is also influenced by innate, possibly genetic differences between individual bees. These differences may reflect the ability shown by some strains of bee to resist paralysis better than others (Rinderer et al., 1975).

The results suggest that CPV adopts a flexible packaging arrangement in which individual RNA species may be packaged separately or various combinations of one or more molecules of the genome components may be packaged together. When large amounts of RNA 3 (a, b and c), but only small amounts of RNAs 1 and 2, are formed it is possible that the individual RNA species are packaged separately (probably in multiple copies in the case of RNA 3) to produce short particles; it appears difficult to form the longer particles in these conditions. In contrast when larger amounts of RNAs 1 and 2, together with smaller amounts of RNA 3, are formed, the RNAs may be packaged together to produce long particles and also separately to produce short particles. In such a situation the overall ratios of the RNA species in long and short particles could be similar, as found for one CPV preparation by Overton et al. (1982). Virions containing all the genome segments would be expected to be more infective than those in which the genome segments are encapsidated separately, particularly at low multiplicities of infection. The finding that long particles are more infective than short ones (Bailey, 1976) is therefore consistent with our suggestions for the packaging arrangements of CPV RNAs.

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