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NOTES

Polyamines Contained by Two Densonucleosis Viruses

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Densonucleosis viruses 1 and 2 both contain the three polyamines putrescine, spermidine, and spermine in complete virus particles but not in their respective top components. The polyamines, with spermidine predominant, comprise 1.41% of the virus particle by weight, which is sufficient to neutralize 26% of the single-stranded DNA contained within the particles.

Polyamines are organic polycations which bind with polynucleotides, and they are associated with a variety of DNA and RNA viruses pathogenic to eukaryotic cells (1, 2, 4, 5, 6, 8 10). In an investigation of the DNA contained by two closely related densonucleosis viruses (DNVs) (candidate insect parvoviruses), it became apparent that the difference in molecular weight between complete virus particles and their "top components"—2.2×10^6—exceeded the estimated size (1.95×10^6) of the single-stranded DNA contained by the two viruses (7). Since the polypeptide composition of the virus particles and their homologous top components is identical (D. C. Kelly, unpublished data), it was probable that the virus particles contained additional material specifically bound to the DNA. We have found that polyamines are specifically associated with DNV particles.

The virus particles and top components (particles devoid of nucleic acid) were prepared as described in the accompanying article (7) from *Galleria mellonella* in the case of DNV1 and from *Aglais urticae* for DNV2. Polyamines were extracted from the virus components with perchloric acid essentially as described by Bachrach et al. (2). A 1-ml portion of 0.1 M NaCl-0.7-mM phosphate buffer (pH 7.0) containing 1 mg of viral protein was acidified with 110 μl of 70% perchloric acid, and the acid-insoluble proteins were removed by centrifugation at 1,000 × *g* for 10 min. A 200-μl portion of the supernatant was mixed with 18.5 mg of Na_2CO_3 and then with 400 μl of dansyl chloride (Sigma Chemical Co. Ltd., London; 30 mg/ml, in acetone). The mixture was then incubated at room temperature for 17 h. The dansyl derivatives were extracted into 250 μl of benzene. Samples (20 to 50 μl) of the extract were applied to silica gel thin-layer chromatography plates (silica gel 60, without fluorescent indicator, 250 μm thick; Merck, Darmstadt, West Germany) which had been prewashed with ethyl acetate-cyclohexane (2:3 vol/vol). The polyamines were then separated in the ethylacetate-cyclohexane solvent. The quantity of polyamines on each plate was determined by scanning negative photographs of the plates in a Joyce-Loebl microdensitometer, which was calibrated with standards photographed under identical conditions at the same time.

Figure 1 shows the resolution of polyamines contained by DNV particles and top components. The quantity of polyamines extracted from these viral components is shown in Table 1. These data show that polyamines are associated only with DNV particles and not with top components, indicating that the polyamines are probably specifically associated with the viral DNA and that they are not adventitiously bound to the capsid protein. Spermidine is the major polyamine contained by both viruses. No cadaverine was detected in the viruses. The relative amounts of the three polyamines from both viruses were virtually identical, despite their origin in different experimental hosts. The presence of polyamines (1.41%, wt/wt) in the virus particle accounts for a part (if not all) of the discrepancy between the size of the DNV single-stranded DNA contained by the virus particle and the difference in molecular weight between the virus particles and their top components.

When DNV particles are thermally denatured and the denaturation is monitored as altering absorbancy at 260 nm, the annealed +/− single strands (created when the virus particles disrupt at about 55 to 60°C) denature at a ther-

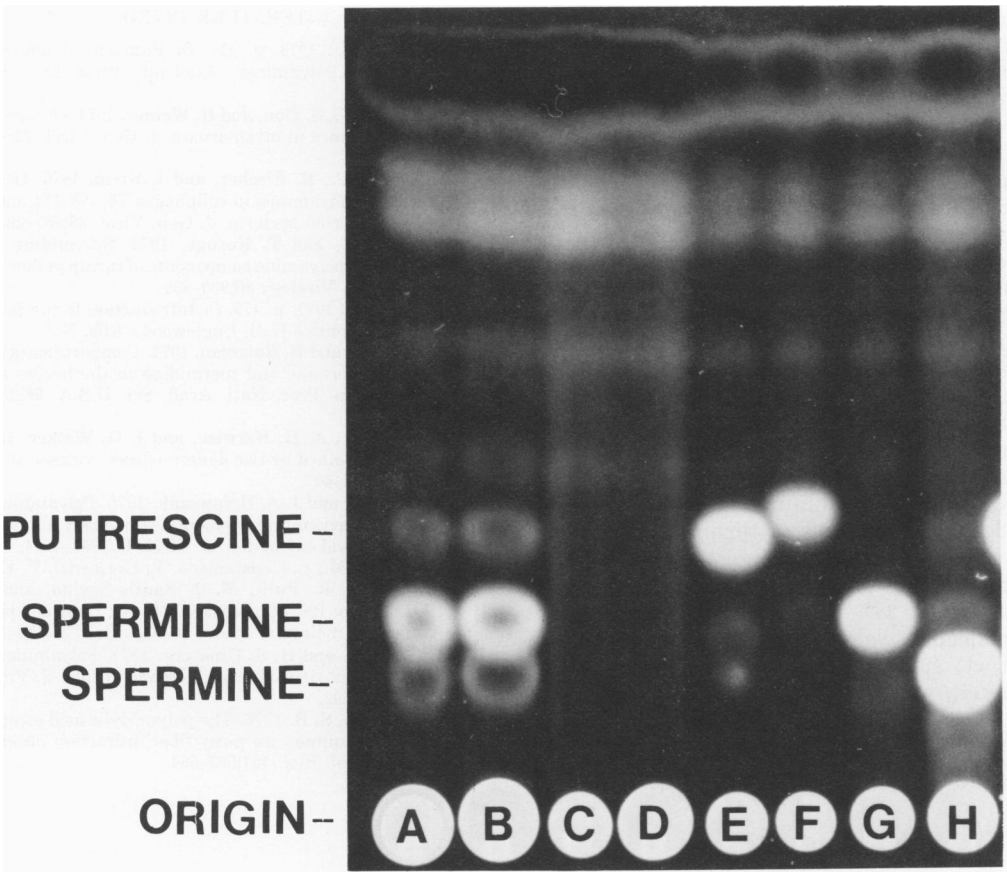

FIG. 1. *Thin-layer chromatogram of the polyamines extracted from DNV particles and top components together with standards. (A) DNV1 virus particles; (B) DNV2 virus particles; (C) DNV1 top components; (D) DNV2 top components; (E) putrescine; (F) cadaverine; (G) spermidine; (H) spermine. (A), (B), (C), and (D) represent 50-μl extracts equivalent to 33 μg of viral protein; (E), (F), (G), and (H) represent 20-μl extracts equivalent to 0.0132 μmol of polyamine.*

TABLE 1. *Polyamine content of DNV particles and top components*

Polyamine	Polyamine content (μg/mg of viral protein)			
	DNV1 VP[a]	DNV2 VP	DNV1 TC	DNV2 TC
Spermine	0.64 ± 0.02[b]	0.62 ± 0.02	0	0
Spermidine	20.08 ± 1.00	20.10 ± 1.00	0	0
Putrescine	1.67 ± 0.07	1.70 ± 0.07	0	0
Cadaverine	0	0	0	0

[a] Virus component. VP, Virus particles; TC, top component.

[b] Mean ± standard error estimated from four determinations.

mal denaturation temperature about 2°C higher than that of purified double-stranded DNV DNA (7). Polyamines stabilize double-stranded DNA (9) and elevate the thermal denaturation temperature of double-stranded DNA (1, 5); therefore, the presence of polyamines in the disrupted virus particle suspension at an effective concentration of about 10^{-4} M would explain this elevated thermal denaturation temperature.

The quantity of polyamines contained by DNV particles is equivalent to 10 molecules of spermine [$H_2N(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$], 450 molecules of spermidine [$H_2N(CH_2)_3NH(CH_2)_4NH_2$], and 62 molecules of putrescine [$H_2N(CH_2)_4NH_2$] per particle and is sufficient to neutralize about 25.7% of the 5,890 phosphate groups contained by the single-stranded DNV DNA genome. This neutralization may aid the condensation of the single-stranded DNA, by promoting base stacking, into a compact form suitable for encapsidation,

and this may aid the separation of complementary strands of DNA prior to their package within particles. We have previously shown that the single-stranded DNA contained by DNV particles has limited secondary structure (7), and polyamines are known to promote base stacking of polynucleotides (11).

Studies on polyamines associated with DNA viruses pathogenic to vertebrates are restricted to viruses with double-stranded genomes (6, 8, 10), and no reports of polyamines associated with other parvoviruses have been made, although it is claimed that the bacterial "parvovirus" ϕX174 contains sufficient polyamines to neutralize 0.5% of the ϕX174 genome (3). Eukaryotic viruses with single-stranded RNA genomes appear to have higher polyamine contents, sufficient to neutralise 30 to 40% of the RNA phosphate contained by myxoviruses (2), and 20% in the case of turnip yellow mosaic virus (a tymovirus). Interestingly, the icosahedral turnip yellow mosaic virus, which contains about 36% (wt/wt) single-stranded RNA of molecular weight 2×10^6, possesses about 1% (wt/wt) spermine and 0.04% (wt/wt) spermidine (4), which is comparable to DNV.

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