Coiled Structure of the Nucleocapsid of Avian Myeloblastosis Virus

(Accepted 9 June 1970)

Little is known about the structure of the nucleocapsid of the RNA oncogenic viruses. Although the nucleocapsid can be observed within virus particles or after isolation from them in negatively stained preparations, subunit arrangement has not been resolved in this group of viruses. While the disruption of the virus envelope of myxoviruses results in a release of the helical nucleocapsid which contains the RNA (Fraenkel-Conrat, 1968), similar disruption of RNA oncogenic viruses by tween + ether or deoxycholate seems to be incomplete, for the method apparently yields intact nucleocapsids (Thé & O'Connor, 1966; Sarkar & Moore, 1968; Calafat & Hageman, 1969).

This communication describes a method for the disruption of AMV with Brij 58 and deoxycholate which yields nucleoids of loosely coiled structure. An approach to the isolation of the nucleocapsid components of AMV was suggested by the method of Godson & Sinsheimer (1967) for lysis of Escherichia coli cells.

Pellets of avian myeloblastosis virus (AMV strain BAI-A: Fig. 1a, b), partially purified from leukaemic chicken plasma by differential centrifugation, were suspended in distilled water containing a final concentration of 0.05% (w/v) of deoxycholate (DOC) and 0.05% (w/v) of Brij 58 freshly dissolved. After 1 hr at 37°, the suspension was layered on to a 5 to 40% (w/v) potassium citrate gradient in 0.024 M-MgCl₂ and 0.034 M-CaCl₂ and centrifuged at 35,000 rev./min. for 210 min. in a Spinco SW 39 rotor. Two bands formed about midway down the gradient and were collected and fixed with 1% glutaraldehyde. The preparations were dialysed against distilled water before the samples were applied to a carbon coated grid. Phosphotungstic acid 2% (pH 7) was used for negative staining.

Examination on a Philips 300 electron microscope revealed that the pooled material from the bands consisted of nucleocapsids. Once DOC and Brij had disrupted the AMV particles, nucleoids were freed almost completely from virus membranes and intact virus particles by density centrifugation. A well-defined ‘inner envelope’ surrounded the intact nucleoids. The nucleoids were spherical with a diameter of 100 to 160 nm. (Fig. 1c). The difference in size from that of the core in complete virus might be due to the hypotonicity of the medium.

Swelling of the nucleoids assisted the resolution of an internal granular, organized structure. Three main structures were observed: (a) an annular form (Fig. 1d); (b) a loose coil or volute (Fig. 1e); (c) an unrolled form with a diameter of 30 to 70 nm and a length of 0.5 to 1 μm. (Fig. 1f). The inner envelope or fragments of envelope may be associated with any of these forms.

No regular repeating structures are seen which could be termed helical or compared in any way with myxovirus nucleocapsids. We cannot exclude that any structure with a labile outer membrane may distort under hypotonic conditions when negatively stained to produce rings, scrolls and linear forms which may or may not be related to the structure of the original and undistorted form. However, the structures observed after fixation with glutaraldehyde have particular morphological characteristics which, in our opinion, seem to be related to the ultrastructure of the original virions.

The possibility was examined that the structures observed were derived from mycoplasma. Samples of leukaemic plasma from five different passages of AMV in chickens were kindly
Fig. 1. Abbreviations: e.v., envelope of the virus particle; e.n., envelope of the nucleoid; n., nucleoid.
(a) Partially purified avian myeloblastosis virus (diameter of the virus particle 150 nm.).
(b) The particle of AMV (e.v.) with its characteristic projections.
(c) The envelope of the nucleoid (e.n.) remained after removal of the virus envelope by the DOC-Brij treatment (diameter 160 nm. or 140 nm. without the envelope).
(d) Annular form of the nucleocapsid (diameter 220 nm.).
(e) Volute form of the nucleocapsid (width 35 nm., length 700 nm.). Fragments of the inner envelope are indicated by arrows.
(f) Unrolled form of the nucleocapsid (30 by 600 nm). Large fragments of the inner envelope are indicated by arrows.
examined by Professor Paul Tournier for the presence of mycoplasma. Mycoplasma broth and mycoplasma agar (Hayflick, 1965) were used. Mycoplasma could not be detected in either broth cultures or agar plates incubated aerobically or anaerobically.

The DNA content of the nucleoids isolated from [3H]thymidine-labelled virus was determined by a modification of the method of Burton (L. Harel, personal communication); protein was estimated by the method of Lowry (1951). Less than 1 μg. of DNA (limit of assay) was found for 188 μg. of protein. Based upon the specific radioactivity of the DNA of the leukaemic cells synthesizing the virus, the radioactivity recovered in the nucleoids corresponded to 0.05 μg. of DNA. This DNA/protein ratio is therefore 1/170 of that expected for mycoplasma.

In addition, particles morphologically similar to mycoplasma were never observed by electron microscopy in purified preparations of AMV or in the sections of chicken fibroblasts infected with leukaemic chicken plasma or purified AMV.

In immunodiffusion tests, the purified nucleocapsids gave one precipitin line with rabbit serum prepared against tween-ether-treated AMV (Lacour, Weiler & May-Levin, 1966) and there was an antigenic identity with AMV group-specific antigen.

Incubation of the nucleocapsids with 0.1% trypsin (Armour crystallized) at 37°C modified their morphology within 30 min. No alteration in the structure of the volutes occurred after incubation with 0.1% RNase for 3 hr at 37°C. Incubation with ether did not destroy the volutes within 20 min. In one experiment where [3H]uridine-labelled virus was used, 70% of the radioactivity was recovered in the nucleocapsids when they were isolated by the method described. This indicates that the nucleocapsid contains the virus RNA.

Single-stranded RNA, with a molecular weight of 10^7 daltons, has been obtained from AMV (Harel et al. 1965). By electron microscopy (Granboulan, Huppert & Lacour, 1966), the modal length of the molecules is 8.7 μm. and the 95% fiducial limits of the mean length 8.1 to 8.5 μm. Further investigation is necessary to define the arrangement of this heavy RNA within the coiled nucleocapsid of AMV: experiments are in progress to determine its biochemical and biophysical properties.

We are grateful to Dr W. Bernhard for discussions and encouragement, to Dr C. Friend for guidance in preparation of the manuscript, and to Liliane Moulart and Mr T. Huynh for capable assistance.

Travail dédié à la mémoire de Nicole Granboulan.

Groupe de Recherches No. 8
Centre National de la Recherche Scientifique
(Department of Immunology)
Department of Electron Microscopy
Institut Gustave-Roussy
94-Villejuif (France)

FANNY LACOUR
A. FOURCADE
C. VERGER
E. DELAIN

REFERENCES
Short communications


(Received 9 February 1970)