Isolation of Non-cubical Ribonucleoprotein from Inkoo Virus, a Bunyamwera Supergroup Arbovirus

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The Bunyamwera supergroup is the largest serogroup of arboviruses. Its members are distributed all over the world and some are medically important. Little is known of the structure and biochemical properties of these viruses. By electron microscopy, partly disintegrated virus particles of two members of this supergroup (Batai and Inkoo) have been reported to liberate coiled structures interpreted as being helical nucleoprotein (von Bonsdorff, Saikku & Oker-Blom, 1969). In this study the nucleoprotein has been isolated from purified virus particles of the Inkoo virus (cryptogram, R/*:*:*:S/E:V/D), a Finnish subtype of the California encephalitis virus (J. Casals, personal communication).

The 4th to 7th tissue culture passages of the Inkoo virus were grown in BHK 21/WI-2 cells (Vaheri et al. 1965). Cell monolayers in Roux bottles were infected with a multiplicity of 0.3 to 6 p.f.u./cell. The virus was grown in Eagle's minimum essential medium containing 0.2% bovine albumin in the presence of 1 μc/ml. of [3H]-uridine or [3H]-leucine added at the time of infection (Radiochemical Centre, Amersham, Buckinghamshire, specific activities 25 c/m-mole and 1 c/m-mole, respectively). After 24 hr the medium was collected, clarified by low-speed centrifugation and concentrated by vacuum dialysis. The concentrate was layered on to a three-phase cushion of 20, 30 and 60% (w/w) sucrose in TES buffer (0.05 M-tris-HCl, 0.001 M-EDTA, 0.1 M-NaCl) pH 7.5. After centrifugation at 80,000 g for 3 hr the virus was recovered on the lowest cushion. Further purification was achieved by sedimenting

![Graph](image-url)

**Fig. 1.** CsCl density gradient analysis of uridine-labelled Inkoo virus degraded with Nonidet P-40. [3H]-uridine-labelled virus was treated with 1% Nonidet P-40 for 30 min. in an ice bath and was mixed with CsCl in TES-buffer, pH 8.5, to give an initial density of 1.34 g./ml. After centrifugation for 24 hr at 4°C and 40,000 rev./min. in a Spinco SW 50 rotor, fractions were collected and their radioactivity in Bray's solution (○---○) and haemagglutination activity (○-○-○) were determined. Densities were measured by weighing 100 μl. samples.
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the virus in a linear 20 to 60 % (w/w) sucrose gradient at 80,000g for 3 hr. The only visible and sharp band coincided with virus infectivity, radioactivity and haemagglutination activity peaks; electron microscopy revealed spherical virus particles similar to those described earlier (von Bonsdorff et al. 1969).

The purified virus was treated with 1 % Nonidet P-40 (Shell Chemical Co.) for 30 min. in an ice bath and then analysed by CsCl equilibrium centrifugation. All [3H]-uridine label was consistently recovered at a density of 1.312 to 1.319 g./ml. (Fig. 1). Of the total [3H]-leucine label about 40 % was found at the same density level, the rest banded at the top of the gradient with all haemagglutination activity (Fig. 2). All label of the respective untreated virus preparations was recovered at the top of the gradient (density about 1.20 g./ml.).

Electron microscopy of the fractions with density of 1.31 to 1.32 g./ml. revealed coiled strands 2 to 3 nm. thick (Fig. 3). Some of these could be followed for at least 1 μm. The strands often showed a rather regular undulation suggesting unwound or stretched helices. In tightly coiled regions diameters between 7 and 10 nm. were determined. However, at these sites no clear helical arrangement was discernible.

The ribonucleoprotein of the Inkoo virus may therefore be helical, and its density in CsCl, 1.31 to 1.32 g./ml., is within the limits reported for helical nucleoproteins of animal RNA viruses (rhabdo- and myxoviruses, Sokol et al. (1969), Wagner et al. (1969), Blair & Duesberg (1970)). The morphology of the isolated Inkoo virus nucleoprotein resembles that of rhabdoviruses (Sokol et al. (1969), Wagner et al. (1969)) and Uukuniemi virus, a tick-borne arbovirus (von Bonsdorff et al. (1969), Saikku, von Bonsdorff & Oker-Blom (1970), Pettersson et al. (1971)), in its tendency to uncoil freely.

These findings suggest that Inkoo virus, and possibly the whole Bunyamwera supergroup of arboviruses, cannot be included in the proposed togavirus group.
Fig. 3. Electron micrographs of Inko virus nucleoprotein isolated from degraded virus by CsCl gradient centrifugation. For electron microscopy the preparations were dialysed against TES-buffer and negatively stained with 1% sodium phosphotungstate, pH 6.4.

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Department of Virology
University of Helsinki
SF 00290 Helsinki 29
Finland

P. Saikku
C. -H. von Bonsdorff
M. Brummer-Korvenkontio
A. Vaheri

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