Self-assembly of the JC virus major capsid protein, VP1, expressed in insect cells

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The major capsid protein of human polyomavirus JC virus, VP1, has been cloned into a baculovirus genome and expressed in insect cells. The VP1 protein was expressed in the cytoplasm and transported into the nucleus. It was then purified by a sucrose cushion and CsCl density gradient centrifugation to near homogeneity. Electron microscopy showed that isolated recombinant VP1 protein self-assembled into a capsid-like structure similar to the natural empty capsid. Both chelator (EDTA) and reducing agent (DTT) are required to disrupt the capsid structure into the pentameric capsomeres, as demonstrated by haemagglutination assay and electron microscopy. These results suggest that JC virus VP1 can be transported into the nucleus and self-assembled to form capsid-like particles without the involvement of the viral minor capsid proteins, VP2 and VP3. In addition, metal ions and disulphide bonds appear to be important in maintaining the integrity of the viral capsid structure.

JC virus (JCV) is a human polyomavirus which belongs to the papovavirus family. The human polyomaviruses, including JCV and BK virus (BKV), are widely spread in the human population (Walker & Frisque, 1986). By the lytic infection of oligodendrocytes, JCV may cause a fatal demyelinating disease, progressive multifocal leukoencephalopathy, in immunocompromised individuals (Padgett et al., 1976). Furthermore, the latency of JCV in normal human brains (White et al., 1992) is speculated to induce a focal demyelinating disease such as multiple sclerosis (Stoner, 1993). The mechanisms of cell transformation by JCV are currently under intense investigation. It has been demonstrated that the large tumour antigen of JCV can bind the retinoblastoma protein (Dyson et al., 1990) and the p53 protein (Mandl & Frisque, 1986), causing transformation of hamster cell lines. In addition, JCV is also able to transform human amnion cells (Howley et al., 1980) and foetal brain cells (Mandl et al., 1987) in vitro. In animal tumour induction, JCV is able to induce various tumours in the Syrian hamster, owl, and squirrel monkey (reviewed by Zu Rhein, 1983).

The polyomavirus virion contains three capsid proteins (VP1, VP2, VP3) and a viral minichromosome. Few studies have focused on the biological functions of JCV structural proteins, partly due to the difficulty in propagating the virus in cell culture (Feigenbaum et al., 1987). The biological properties of insect cells enable them to express structural proteins of murine polyoma virus similar to those derived from in vivo cultured virions (Montross et al., 1991; Forstova et al., 1993). In this report, the major capsid protein of human polyomavirus JCV, VP1, has been expressed in insect cells and characterized.

In this study, the JCV VP1 gene was cloned into the baculovirus transfer vector pBacPAK8 (Clontech) to generate pBJCV1. The pBJCV1 plasmid DNA was cotransfected into Spodoptera frugiperda (Sf21) cells with the baculovirus DNA. Immunoblotting showed that VP1 was expressed at 24 h post-infection (p.i.) and maintained for 120 h. The maximum expression level was at about 72 h p.i. (data not shown).

The intracellular localization of JCV VP1 expressed in insect cells was monitored by indirect immunofluorescence analysis (IFA). The infected Sf21 cells were grown on glass coverslips and fixed with acetone–methanol. The fixed cells were reacted with anti-JCV VP1 antiserum at a dilution ratio of 1:200. A secondary antibody, goat anti-rabbit IgG, conjugated with fluorescein isothiocyanate (FITC; Sigma) was used to detect JCV VP1 in the insect cells. The cells were visualized by
fluorescent microscopy (Zeiss). Our IFA results show that JCV VP1 was expressed and detected in the cytoplasm of insect cells (Fig. 1b) and then transported into the nucleus (Fig. 1c). The infected cells appeared to be lysed at 120 h p.i. (Fig. 1d). The results show that JCV VP1 was synthesized in the cytoplasm and transported, mediated by its own nuclear localization sequence, into the nucleus.

The positive results from the haemagglutination (HA) assay of the cell lysate imply that JCV VP1 can self-assemble to form capsid-like structures in insect cells. Therefore, CsCl density gradient centrifugation was performed to purify the capsid particles. Insect cells (Sf21) were infected with recombinant baculovirus carrying the JCV VP1 gene, at a titre of 15 p.f.u. per cell. Cells were harvested by centrifugation at 72 h p.i. The cell pellet was resuspended in a lysis buffer (50 mM NaCl, 0-01 mM CaCl₂, 10 mM Tris–HCl pH 7-4, 0-01% Triton X-100), and disrupted by sonication. Cell debris was removed by centrifugation at 10 000 g for 20 min. The supernatant was subjected to a 20% sucrose cushion (10 ml) centrifugation at 35 000 r.p.m. for 3 h in a SW 41 Ti rotor (Beckman). The pellet was resuspended in Tris pH 7-4 buffer and diluted to a density of 1·20 g/cm³, then layered onto a five-step CsCl velocity gradient consisting of 2 ml of 1·20, 1·25, 1·29, 1·32 and 1·35 g/cm³ CsCl solution. After centrifugation at 35 000 r.p.m. for 36 h, every fraction was assayed by haemagglutination of type O human red blood cells and density determination (refractometer from Nippon Optical Works). The results show that the peak of HA activity was between fractions 22 to 28 (Fig. 2a). The buoyant densities of the peak area are from 1·28 to 1·30 g/cm³. The presence of VP1 protein within the HA peak was determined by SDS–PAGE (Fig. 2b). Fraction 26 showed the highest HA activity (Fig. 2a) and contained the largest amount of VP1 protein (Fig. 2b, lane 6). Particles purified in gradient fractions (Fig. 2b) were viewed by electron microscopy (Fig. 2c) with a JEOL 2000-CX electron microscope operated at 80 kV. The capsid-like particles appeared nearly homogeneous, with a diameter of 45 nm. The particles were uniform in size and resembled empty capsids (Fig. 2c).

The subspecies of JCV VP1 expressed in insect cells were also analysed by isoelectric focusing (Bolen et al., 1981) and SDS–PAGE, and identified by Western blot. The results show that recombinant JCV VP1 expressed in insect cells was modified to form six isoelectric species. Isoelectric points of the six species ranged from 5·8 to 6·4 (data not shown).
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Metal ions and disulphide bonds have been shown to play important roles in maintaining the integrity of the capsid structure of murine polyoma (Brady et al., 1977) and simian virus 40 (SV40) (Christiansen et al., 1977). When adding either EDTA (10 mM) or DTT (3 mM final concentration) to the JCV capsid, the capsid structure did not dissociate, as determined by HA assay (Fig. 3a) and electron microscopy (data not shown). However, when the capsid was treated with both EDTA and DTT, it dissociated to form capsomeres (Fig. 3b) and lost its HA activity (Fig. 3a). These results indicate that both metal ions and disulphide linkages are essential for maintaining the capsid integrity of JCV.

The major capsid protein of human polyomavirus JCV, VP1, was expressed in insect cells. After being synthesized in the cytoplasm, JCV VP1 was transported into the nucleus. When the amino acid sequence of JCV VP1 was examined, it was found that the first 12 amino acids at the N terminus of the protein form a basic amino acid stretch, MAPTKKRGERKD, which is similar to the nuclear localization signals (NLS) of murine polyoma VP1, MPRKKSVCSC (Chang et al., 1992; Moreland & Garcea, 1991) and SV40 VP1, MAPTKRKSGCPGAAPKKPK (Ishii et al., 1996). Therefore, the putative NLS of JCV VP1 located at the N terminus may be responsible for transporting JCV VP1 into the nucleus for viral assembly.

Previously, Montross et al. (1991) have shown that murine polyoma VP1 is able to form capsid-like structures in the nuclei of Sf9 insect cells. In this report, we found that JCV VP1 can self-assemble to form empty capsid-like particles similar to those of murine polyoma VP1 expressed in insect cells (Montross et al., 1991), although JCV VP1 expressed in insect cells has been found to have DNA-binding activity (data not shown). In contrast, when murine polyoma VP1 cotransfected with the minor capsid proteins, VP2 and VP3, into insect cells, the capsid-like particles resembled the complete virion particles (Forstova et al., 1993). Therefore, encapsidation of DNA during viral assembly may require the minor capsid proteins, VP2 and VP3, for modulation (Forstova et al., 1993).

It is known, based on previous reports of murine polyoma virus, that the VP1 molecule is not a single protein entity, but can be separated into six distinct species (A to F) (Bolen et al., 1981). Bolen et al. (1981) also showed that the haemagglutination-inhibiting portion of polyoma-specific antisera reacted with species D and F, and the neutralizing fraction of
the same antisera reacted with species E. The results indicate that species E is required for attachment to and infection of mouse kidney cells. JCV VP1 was found to be post-translationally modified in insect cells and formed six isoelectric species. The functions of each species of JCV VP1 need to be further determined.

The three-dimensional structure of simian polyomavirus SV40 VP1 has been determined by X-ray crystallography (Liddington et al., 1991), and shows that the 72 pentamers of viral VP1 have identical conformation, except for the C-terminal arms of their subunits. The divalent cation Ca$^{2+}$ is an integral part of polyomaviruses, plays a major role in stabilizing the intact virion structure (Brady et al., 1977) and is likely to be involved in the processes of viral uncoating and assembly. In the SV40 VP1 molecule, the Ca$^{2+}$-binding sites have been predicted to reside on the C arm, at aspartic acid 266, by site-directed mutagenesis in vitro (Haynes et al., 1993). There are no disulphide bonds found in the SV40 crystal structure (Liddington et al., 1991), but reducing agents are required to dissociate polyomavirus (Brady et al., 1977) and SV40 (Christiansen et al., 1977). Disulphide bonds may occur at cysteine 104, between CD loops of neighbouring capsomeres (Liddington et al., 1991). In this study, both chelator and reducing agent were necessary to dissociate JCV capsid particles, which indicates that metal ions and disulphide bonds are involved in maintaining virus integrity. The baculovirus-expressing system will be employed for further characterization of VP1, the major capsid protein of JCV, to help us understand the mechanism(s) of viral assembly. In addition, it may also be possible to develop a vaccine and a clinical diagnostic reagent for JCV by using the recombinant capsid particles expressed in insect cells.

References


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Fig. 3. Dissociation of JCV capsid-like particles by EDTA and DTT. (a) JCV capsid-like particles purified by a CsCl density gradient (control) were incubated with 10 mM EDTA and/or 3 mM DTT for 1, 2, 3 and 4 h. Capsid integrity was indicated by haemagglutination activity. (b) Electron microscopy of the capsomeres dissociated from capsid-like particles by the addition of EDTA and DTT. Bar = 100 nm.
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