Structural Organization of Murine Intracisternal A Particles

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Isolated murine intracisternal A particles have a distinctive set of structural properties as compared to the recognized oncogenic ribonucleic acid tumor viruses. A particles consist of two closely approximated concentric shells. The outer shell is largely, but not entirely, susceptible to treatment with detergents such as deoxycholate and Triton X-100. The inner shell is resistant to these agents and is stabilized against solubilization by sodium dodecyl sulfate as a result of disulfide bonding between its constituent proteins. The major A particle structural protein (molecular weight approximately 70,000) is contained in the inner shell. A protein component with a molecular weight of about 80,000 may be preferentially concentrated in sites of firm attachment between the inner and outer shells.

Intracisternal A particles are present in a variety of normal and neoplastic mouse tissues (13). They consist of two concentric shells surrounding a relatively electron-lucent core, range in diameter from 70 to 100 nm, and form by budding at the membrane of the endoplasmic reticulum. After formation, they remain in the cisternae. Because of their morphological appearance, they have been included in a classification of oncogenic ribonucleic acid (RNA) viruses (11). Although it has not yet been possible to demonstrate biological activity in association with such particles (4, 5, 7, 10), they may reflect the expression of a viral genome which is vertically transmitted in mice (13). These intracisternal A particles are distinct from the intracytoplasmic A particles which are thought to be the precursors of the mouse mammary tumor virus (11).

In a previous publication we have detailed the extraction of intracisternal A particles from transplantable mouse plasma cell tumors (5). Isolated particles were found to contain 80% protein, 14% phospholipid, and 5 to 6% RNA. At least 40% of the RNA was contributed by contaminating ribosomal material. Most of the phospholipid was present in the outer shell.

Subsequently (4), we showed that intracisternal A particles from several different plasma cell tumors and from neuroblastoma tissue culture cells all contain a major structural protein with an apparent molecular weight of approximately 70,000. When the size distribution of A particle proteins was compared with those of murine leukemia viruses and mammary tumor virus, marked differences were demonstrated. Rabbit antiserum which reacted with the major protein of the A particle was used in immunodiffusion and complement fixation assays. Numerous mouse tumors known to contain intracisternal A particles all reacted with this antiserum. Samples of murine leukemia virus and mammary tumor virus showed no reactivity, nor did A particles react with antisera against leukemia virus antigens (gs-1 and gs-3).

It has now been established that there is a deoxyribonucleic acid (DNA) polymerase activity associated with intracisternal A particles that transcribes poly rA in the presence of oligo dT or poly dT primer (12). This enzyme activity differs in several of its properties from the DNA polymerase associated with Rauscher murine leukemia virus, thus demonstrating another apparently distinctive characteristic of intracisternal A particles.

The present studies were undertaken to define more precisely some of the structural properties of intracisternal A particles and to further compare this system with known oncogenic RNA viruses.

MATERIALS AND METHODS

Tumor. Plasma cell tumor MOPC-104E was maintained by subcutaneous transplantation in female BALB/c mice. The tumor line was obtained from Michael Potter of the National Cancer Institute.

A particle isolation. A particles were prepared by centrifugation through a cushion of 48% (w/v) sucrose according to the standard isolation procedure previously reported (5). The pellet was suspended in 10 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 8.0, and used for subsequent detergent extraction of A particles. The protein electrophoretic pattern for this preparation was indistinguishable from that of gradient purified A particles.

Detergent treatment. Extraction of A particles with sodium deoxycholate (DOC) was carried out as follows. The A particle suspension (12.8 mg of protein per ml) was thoroughly dispersed by sonic vibration (10 kc) during two 15-sec intervals at 5 C in a Raytheon magnetostriction oscillator, model DF 101, and DOC (10%) was added to a final concentration of 1%. The suspension (0.4 ml) was then layered over a series of 1.6-ml cushions of 24, 48, and 68% (w/v) sucrose in 10 mM Tris-hydrochloride, pH 8.0, in a Lusteroid tube of the Spinco SW50.1 rotor and centrifuged at 50,000 rev/min for 30 min at 5 C. The supernatant fluid above the sucrose cushions contained DOC-solubilized material. The sucrose layers were clear. The DOC-washed A particles were collected at the interface between the 48 and 68% sucrose cushions where they formed a light scattering band.

For further treatment with sodium dodecyl sulfate (SDS), samples of DOC-washed particles were diluted threefold with 10 mM sodium phosphate, pH 7.2, to a final concentration of 1% SDS. These samples were then layered over 0.2-ml cushions of 20% (w/v) sucrose in Lusteroid tubes (0.6 by 3.3 cm). Using adapters fitting the Spinco SW50.1 rotor, the tubes were centrifuged at 40,000 rev/min for 30 min at 15 C. The supernatant fluids contained SDS-solubilized material. The pellets which contained the SDS-resistant A particle shells were suspended in 10 mM sodium phosphate, pH 7.1.

Electrophoresis. Proteins were analyzed by electrophoresis in 6% polyacrylamide gels containing 6 M urea in a running buffer of 100 mM sodium phosphate, pH 7.1, and 0.1% SDS (6). Gel columns (0.5 by 5.5 cm) were subjected to pre-electrophoresis for 15 min. Fractions in 10 mM sodium phosphate (pH 7.1) were made to 1% SDS and 0.1 M 2-mercaptoethanol, and heated for 1 min at 100 C (4) to reduce disulfide bonds. Samples were immediately loaded on gels and subjected to electrophoresis for 3.5 hr at room temperature with a constant current of 3 ma per gel. The gels were stained by a modification (D. Marciani, *personal communication*) of the method of Fairbanks et al (3). The gels were fixed and stained in 25% isopropanol, 10% acetic acid, and 0.05% Coomassie Blue overnight and destained in 10% isopropanol and 10% acetic acid for 6 hr, followed by a 5% methanol-7% acetic acid soak. The molecular weights of A particle components were estimated by comparing their electrophoretic mobilities with those of known marker proteins.

Electron microscopy. The preparation of materials and the electron microscope techniques are those that have been described previously (5).

RESULTS

A particles were subjected to sequential detergent extraction, and the resultant fractions were monitored by both electron microscopy and gel electrophoresis. For the latter purpose, samples of each fraction were fully solubilized by treatment with 2-mercaptoethanol at 100 C in the presence of SDS.

Intracisternal A particles, as freshly isolated from MOPC-104E myeloma, are shown in Fig. 1. The two concentric shells of the particles have been clearly delineated by the negative stain, with the inner shell width being over three times that of the outer one (Fig. 1, insert). These shells appear to be separated by a narrow space of rather constant dimension. In most instances, phosphotungstic acid has filled the inner core of the particles suggesting that this central area is empty. The protein electrophoretic pattern of the whole A particle preparation is shown in Fig. 4A. The major electrophoretic component (Fig. 4, band 2) has an apparent molecular weight of nearly 70,000, as previously described (4). Other prominent bands in Fig. 4 representing material with apparent molecular weights near 80,000 and 45,000 are designated 1 and 3, respectively. A number of minor bands were also detected; many of these are not apparent in the photographed gel.

The outer A particle shell, derived from the membrane of the endoplasmic reticulum, is sensitive to detergents such as DOC and Triton X-100 (5). DOC-treated particles are shown in Fig. 2. Although the outer membrane has been largely removed, fragments remain adherent to the inner shells (Fig. 2, insert) even after repeated exposures to the detergent. Similar bits of adherent material were observed after treatment with 1% Triton X-100 or Tween-ether (2, *not illustrated*). These fragments were not detectable by electron microscope examination of thin-section preparations of the detergent-treated particles (5).

The electrophoretic pattern of DOC-washed particles (Fig. 4B) was similar in major aspects to that of the untreated fraction, although this detergent is known to solubilize about 25% of the total protein in isolated A particles. The reason for this is apparent from Fig. 4D, which shows that the DOC-solubilized material (examined here at high relative concentration) consisted largely of components whose individual contributions to the original A particle electrophoretic pattern were minimal. An exception was electrophoretic component 1, which appeared as the most prominent band in

this fraction. Calculations based on optical density scans of the stained gels and the relative amounts of material subjected to electrophoresis indicate that 30 to 40% of this component was solubilized by the DOC treatment.

A particle preparations treated sequentially with DOC and SDS (or in other experiments directly with SDS) yielded a particulate fraction with the appearance shown in Fig. 3. These structures were derived from the inner shells which appeared to have flattened on the grid with obliteration of the central space. The enlarged particle diameters may reflect binding of SDS to the inner shell proteins and subsequent binding of water from the aqueous negative stain solution. The particles exhibited a surface granularity with the discernible subunit structures having center-to-center dimensions of about 30 nm; however, no regularity in arrangement was apparent. Fragments of the outer particle shell that had resisted DOC extraction were not observed after exposure to SDS.

The SDS-treated particles were sensitive to the action of disulfide bond reducing agents. Addition of dithiothreitol or 2-mercaptoethanol resulted in solubilization of the inner shell structures in neutral SDS-containing buffer, as judged both by migration of the components in gel electrophoresis (Fig. 4C) and by the disappearance of structures observable by electron microscopy. However, the inner shell protein remained insoluble in Triton X-100, DOC, and Tween-ether, even after treatment with high concentrations of sulfhydryl compounds.

Electrophoretic analysis of the SDS-resistant inner shells (Fig. 4C) showed that component 1 was absent; components 2 and 3 appeared in the same relative amounts as in the original A particle preparation. There was a moderate accentuation of staining in the higher-molecular-weight regions of the gel pattern and the appearance of a new band (Fig. 4, asterisk) in the position appropriate to a dimer of component 2. Other preparations of SDS-treated particles, especially those that had been pelleted at high centrifugal forces, have shown extensive formation of high-molecular-weight aggregates that could not be reversed by reduction in SDS at 100 C.

Figure 4E shows an electrophoretic analysis of the fraction extracted by SDS from A particles that had been previously treated with DOC. Electrophoretic component 1 was by far the major element in the pattern. Very little of component 2 was seen, confirming our original observation (4) that SDS solubilization of the main proteins of isolated A particles was rigorously dependent upon disulfide-bond reduction.

DISCUSSION

The observations described here for particles derived from MOPC-104E myelomas have been confirmed with preparations from a second myeloma line (RPC-20) and from neuroblastoma cells. Thus, these findings outline certain general aspects of the structural organization of murine intracisternal A particles and permit us to consider more clearly their relationship to the C and B type tumor viruses.

The inner shell of isolated A particles is resistant to DOC and Triton X-100 and is stabilized against solubilization by SDS as a result of disulfide bonding between its constituent proteins. The extent of disulfide linkage in the particles as they exist in situ is not yet known; nevertheless, this property in isolated preparations greatly facilitates localization of the various components.

All available evidence indicates that the major structural protein is confined to and largely composes the inner particle shell. Particles derived from MOPC-104E tumors always exhibit at least one additional inner-shell component in the molecular-weight range of 45,000, and some electrophoretic runs resolve another separate band on the leading edge of the major protein. Particles prepared from different sources appear to differ characteristically in the number and proportion of minor electrophoretic components associated with the SDS-resistant inner shell.

The inner shell is not simply enclosed within the outer envelope; rather, these two layers appear to be firmly adherent at many sites (Fig. 2). An 80,000 molecular-weight protein is the most prominent electrophoretic component in detergent extracts of the oxidized particles. As a working hypothesis, we suggest that this protein is localized in the outer particle shell and is concentrated in the more tightly adherent (DOC-resistant) portions of this membrane. The distribution of this component in the endoplasmic reticulum membrane as a whole, as compared to that portion specifically associated with the A particles, is currently under investigation. It is likely, of course, that many proteins of the endoplasmic reticulum are incorporated into the outer particle shell during the budding process. However, local concentrations of specific membrane components could be instrumental in determining sites of particle assembly.
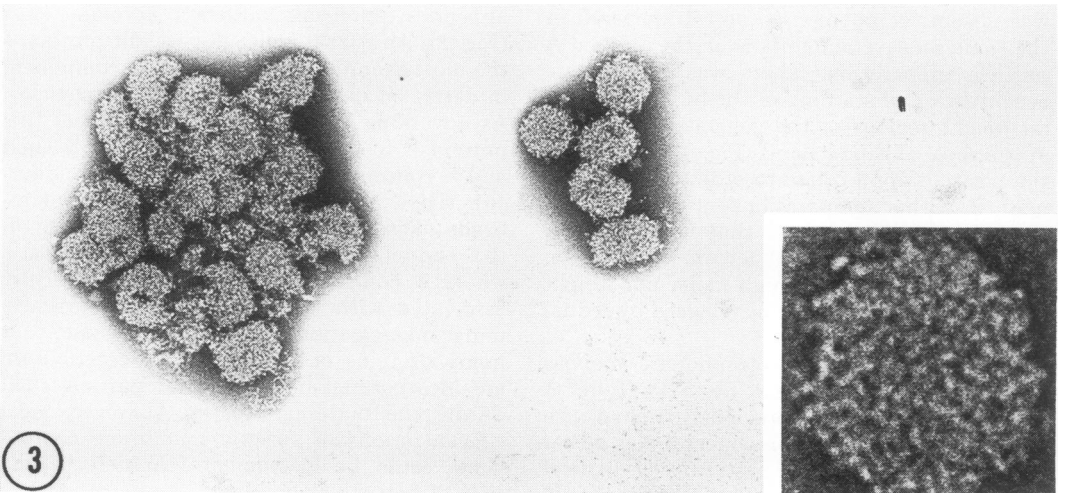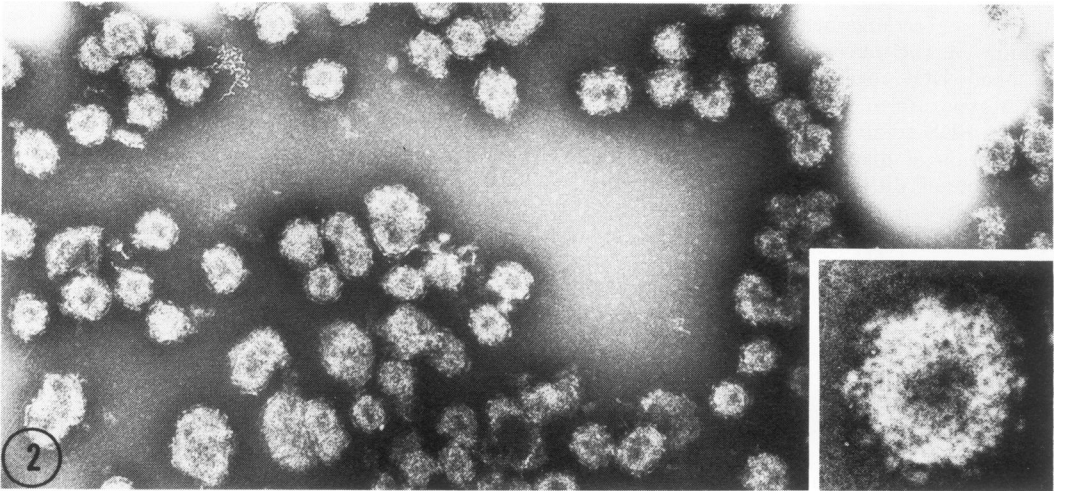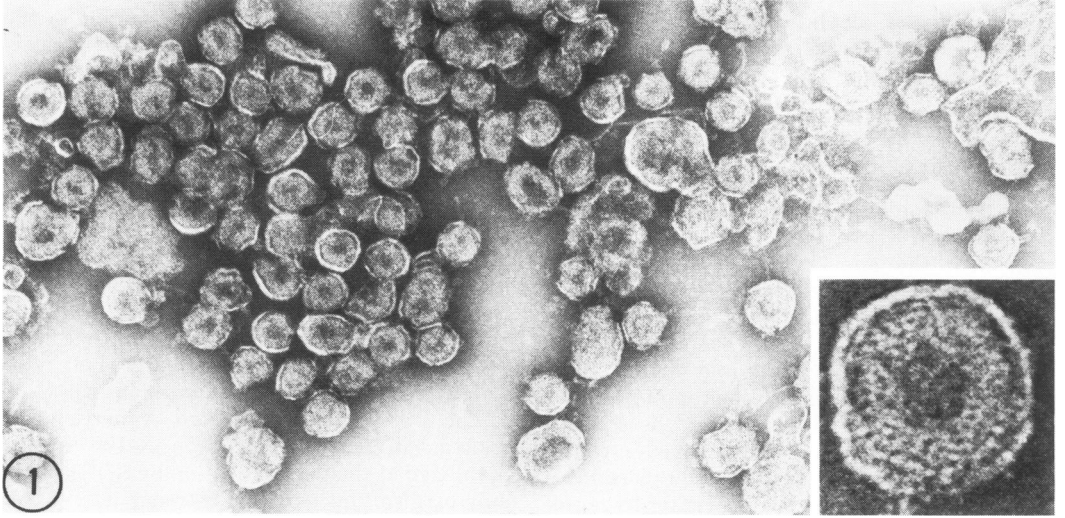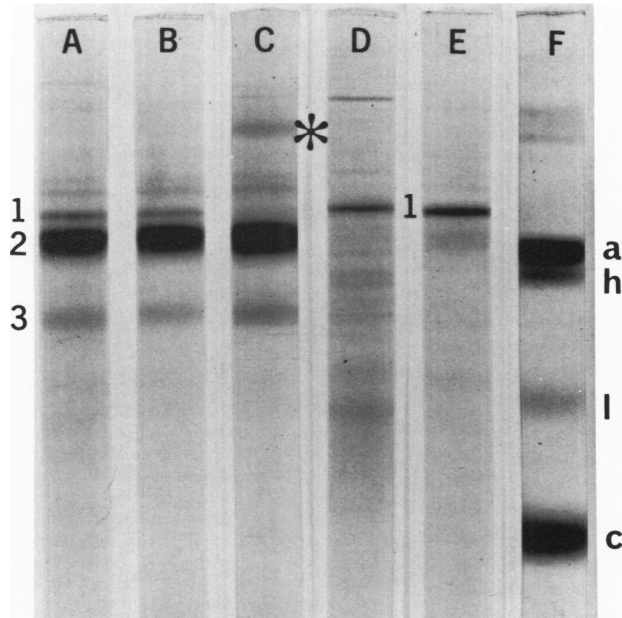
FIG. 4. Gel electrophoresis of isolated MOPC-104E A particles (A), A particles after treatment with DOC (B), A particles after treatment with DOC followed by SDS (C), DOC extract (D), SDS extract of DOC-treated particles (E), protein standards (F), a, Human serum albumin, molecular weight 68,000; h, immunoglobulin G (IgG) heavy chain, molecular weight 55,000; l, IgG light chain, molecular weight 23,500; c, cytochrome C, molecular weight 12,300. All preparations were reduced with mercaptoethanol. Numbers 1, 2, and 3 designate major electrophoretic components. *Denotes a band in a position appropriate to a dimer of component 2.

In collating those common structural features which murine intracisternal A particles share with known oncogenic RNA viruses, it is apparent that both are formed by budding at lipoprotein membranes (although at different intracellular sites), and during this process they acquire an outer shell (A particles) or an envelope (C particles). In both, the major structural proteins are associated with the internal components of the particles, i.e., viral nucleoids and A particle inner shells. It seems well established that the nucleoid of B and C particles contains an intrinsic RNA of high molecular weight (60 to 70S). Recently it has been possible to demonstrate that there are 60 to 70S RNA species associated with intracisternal A particles (J. Virol., in press).

Conversely, the differences between the known oncogenic RNA viruses and A particles are quite marked. A particles lack an outer

FIG. 1. Electron micrograph of a preparation of intracisternal A particles as freshly isolated from MOPC-104E myeloma. Only a few contaminating vesicles are seen. The two concentric shells of the particles have been clearly delineated by the negative stain. In many instances phosphotungstic acid has filled the inner core of the particles, suggesting that this central area contains no more than open molecules which can be easily penetrated. Magnification is approximately 53,000. Insert, The bulk of the particle is contributed by the inner shell which averages about 18 nm in width; the smaller outer shell averages about 5 nm in width. There is a narrow space of rather constant dimension (5 nm) separating the two shells. Magnification is approximately 240,000.

FIG. 2. Electron micrograph of DOC-treated A particles. Although most of the outer shell has been removed, there are external fragments remaining which probably represent remnants of outer shell. The structure of the inner shell remains essentially unchanged. Magnification is approximately 53,000. Insert, Compare appearance of outer shell with that seen in insert of Fig. 1. Magnification is approximately 240,000.

FIG. 3. Electron micrograph of A particle preparation treated sequentially with DOC and SDS. The inner shell of the particle has collapsed with obliteration of the central space. Magnification is approximately 53,000. Insert, Structures derived from inner shell have surface subunits with a center-to-center dimension of about 30 nm. No regularity in arrangement of these subunits is apparent. Magnification is approximately 240,000.

envelope derived from the plasma membrane of the cell. Differences in antigenic and physical properties of the particle proteins and in the characteristics of the particle-associated DNA polymerase activities have been noted elsewhere (4, 12), while the present study suggests other fundamental differences in the organization of the A particles as compared to the extracellular viruses. Thus, a variety of detergents including Triton X-100, DOC, and Tween-ether remove in apparent entirety the envelope of various oncogenic RNA viruses (2) and dissociate certain internal proteins (8, 9); whereas similar treatments fail to remove completely the outer A particle shell and reveal substructures representing quite stable attachments between inner and outer shells. Intermolecular disulfide bonding of the major structural proteins is not a salient feature of the B and C type particles (4), as it is in the case of the isolated A particles.

In considering the characteristics of the intracisternal A particles as they are now known, it may well be that the relative insolubility of the main inner shell protein contributes to the observed lack of biological activity. We know from previous experiments that isolated A particles can be taken up by cells and that they appear in phagocytic vacuoles. However, there has been no evidence for production of new A particle antigens in these cells. If for any reason a successful replicative cycle should require solubilization of the particle proteins, then it becomes readily apparent that a host cell may lack requisite enzymes to facilitate such a process.

In summary, intracisternal A particles are most numerous in neoplastic cells and possess several characteristics which are reminiscent of oncogenic RNA viruses. Their particular structural properties appear to be unique, and it will be important to determine if such properties are in any way related to the existence of a viral genome.

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