LOCALIZATION OF REOVIRUS CELL ATTACHMENT PROTEIN σ1 ON THE SURFACE OF THE REOVIRION USING IMMUNOFERRITIN ELECTRON MICROSCOPY

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Abstract

Purified reovirus type 3 (strain Dearing) was treated with monoclonal anti-σ1 antibody conjugated to ferritin and examined in the electron microscope. Virion-associated ferritin molecules corresponding to locations of the σ1 protein were observed. Electron microsocpy of thin sections of these preparations revealed that ferritin conjugates were localized at the vertices of the viral icosahedron. Reovirus cores possessing spikes and protein σ1, prepared by partial chymotrypsin digestion of intact virions, were similarly treated and examined. Core-associated ferritin molecules were found to be localized at positions corresponding to the tips of the spikes. Such an association was not observed with the cores whose spikes had been removed by alkali treatment. These results provide unequivocal evidence that protein σ1 is present in icosahedrally-distributed locations, representing tips of reovirus core spikes, on the surface of reovirus particles.

Introduction

Considerable research effort has been directed in recent years at defining cell-virus interactions at the plasma membrane level, in particular, those involved in viral attachment.

Although significant advances have been made, in only a few virus systems have specific receptors on the host cell membrane been identified. On the other hand, evidence that specific surface components of certain viruses are involved in the recognition of cellular receptors, though not overwhelming, is nevertheless more secure. The presence of such structures has been implicated in a number of viruses including influenza virus, VSV, poliovirus, adenovirus, polyoma virus and reovirus [1,2].

In the case of reovirus, a single outer capsid protein, σ1 which may exist in either an extended or an unextended state, has been identified as the viral cell attachment protein [3,10,21]. Protein σ1 is also the reovirus hemagglutinin [4] and has been shown to be responsible for type specificity [5,6,7,8], host immune responses [9,10,11,12], as well as tissue tropism and virulence [13].

This protein therefore specifies how reovirus particles interact with host cells and with the host. Binding

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competition studies using various monoclonal antibodies directed against proteins of the outer viral capsid, have concluded that σ1 is located on the surface of the reovirus particles in close juxtaposition to λ2, the principal, if not the sole, component of the icosahedrally arranged reovirus core projections or spikes [14] which penetrate through the outer capsid shell to the particle surface [15].

In this report, anti-σ1 antibody conjugated to ferritin was used in electron-microscopic studies to reveal the location and distribution of the σ1 protein on the reovirus.

Materials and Methods

Monoclonal antibody against reovirus type 3 σ1 was produced from the G5 hybridoma cell line [16] which was originally obtained from Dr. B.N. Fields of Harvard University Medical School and was provided by Dr. P. Lee from Canada.

The antibody was isolated from cell culture supernatants of G5 using a protein-A-Sepharose 4B column as previously described [17]. Purified anti-σ1 IgG in PBS (1 mg/ml) was then conjugated to ferritin (Miles Biochemicals, 6 x crystallized and repurified) using glutaraldehyde according to the method of De Petris and Reff [18]. The purified antibody-ferritin conjugate thus prepared was found to retain the capacity to immunoprecipitate reovirus. Ferritin conjugated to antibody against σ3, the major reovrius outer capsid protein, and to antibody against σ3, the major reovirus outer capsid protein, and to antibody against *Clostridium difficile* toxin (19) were similarly prepared, adjusted to the same concentration as the anti-σ1-ferritin conjugate (by optical density measurement at 440 nm), and used as positive and negative controls, respectively.

For electron-microscopic studies, 50 μg of reovirus type 3 (strain Dearing), propagated and purified as previously described [20], was mixed with 30 μg of the antibody-ferritin conjugate in a total volume of 100 μl in PBS and the mixture was then incubated at room-temperature for 30 minutes. A 5μl aliquot of the mixture was then placed on a 200-mesh formvarcoated grid which had been pretreated with 0.1% BSA.

After 2 minutes, the grid was washed with 10 drops of PBS within a span of 1 minute, and subsequently rinsed with distilled water, dried, and examined under a Zeiss EM 10 electron microscope. Virus samples to be thin-sectioned were prepared first by incubating purified reovirus with the anti-σ1 antibody-ferritin conjugate as described above, followed by immunopreciptation of the treated virus with a 1:50 dilution of anti-σ3 antiserum. Aggregated virus was then pelleted by centrifugation at 12,800 xg for 2 minutes in a microfuge, washed three times in PBS, and fixed with glutaraldehyde (0.25 M in 0.1 M phosphate buffer, PH 7.2) for 1 hour at 4°C. Virus pellets were then washed in PBS, fixed in 1% osmium tetroxide for 2 hours and embedded in Epon 812. Thin sections of these samples were then prepared, stained with 5% uranyl acetate in methanol, and examined under the electron microscope.

Results and Discussion

A preparation of purified reovirus negatively stained with 2% phosphotungstic acid (PH 7.0) is shown in Figure 1A, in which the outer capsid is clearly discernable. For the unstained virus preparations treated with anti-σ1-ferritin conjugates, detailed structure of virus particles could not be resolved. However, virion-associated ferritin molecules were found to be sparsely distributed at positions which may correspond to the vertices of the icosahedral virus particle (Fig. 1B).

That σ1 molecules are probably located at these positions has been previously arrived at through binding competition experiments using a number of monoclonal antibodies directed against proteins of the outer viral capsid [19].

The relatively sparse distribution of anti-σ1-ferritin conjugates on the reovirus is also in sharp contrast with the abundance and the apparently random arrangement of virus-associated ferritin molecules observed in virus samples treated with an anit-σ3-ferritin conjugated whose concentration had been adijusted to that of the anti-σ1-ferritin conjugate (Fig. 1C). This observation is consistent with the previous finding that σ3 is the principal constituent of the outer capsid shell (approximately 900 molecules per virus particle) (17). On the other hand, few virus-associated ferritin molecules were found in control virus samples treated with an equivalent amount of anti-C.difficile toxin-ferritin conjugates (Fig. 1D). An examination of 50 virus particles (chosen at random) of each preparation revealed that, on the average, there were 3.7 and 0.14 associated ferritin molecules per virion for preparations treated with anti-σ1-ferritin and with conjugates, respectively (Table 1).

The association between reovirions and anti-σ1-ferritin conjugates is therefore specific. This was also demonstrated by the observation that pretreatment of virus particles with anti-σ1 antibody prevents such an association (date not shown).

It was observed, however, from Figure 1B and Table 1, that the average number of anti-σ1-ferritin conjugates bound per virion did not even approach the number of sites (twelve) theoretically possible for σ1. While it is possible that not all σ1 molecules on the virion are capable of reacting with the conjugate, it is more likely that this reflects the low concentration of conjugate used. (We avoided using saturating concentrations of the conjugate to prevent extensive viral aggregation which

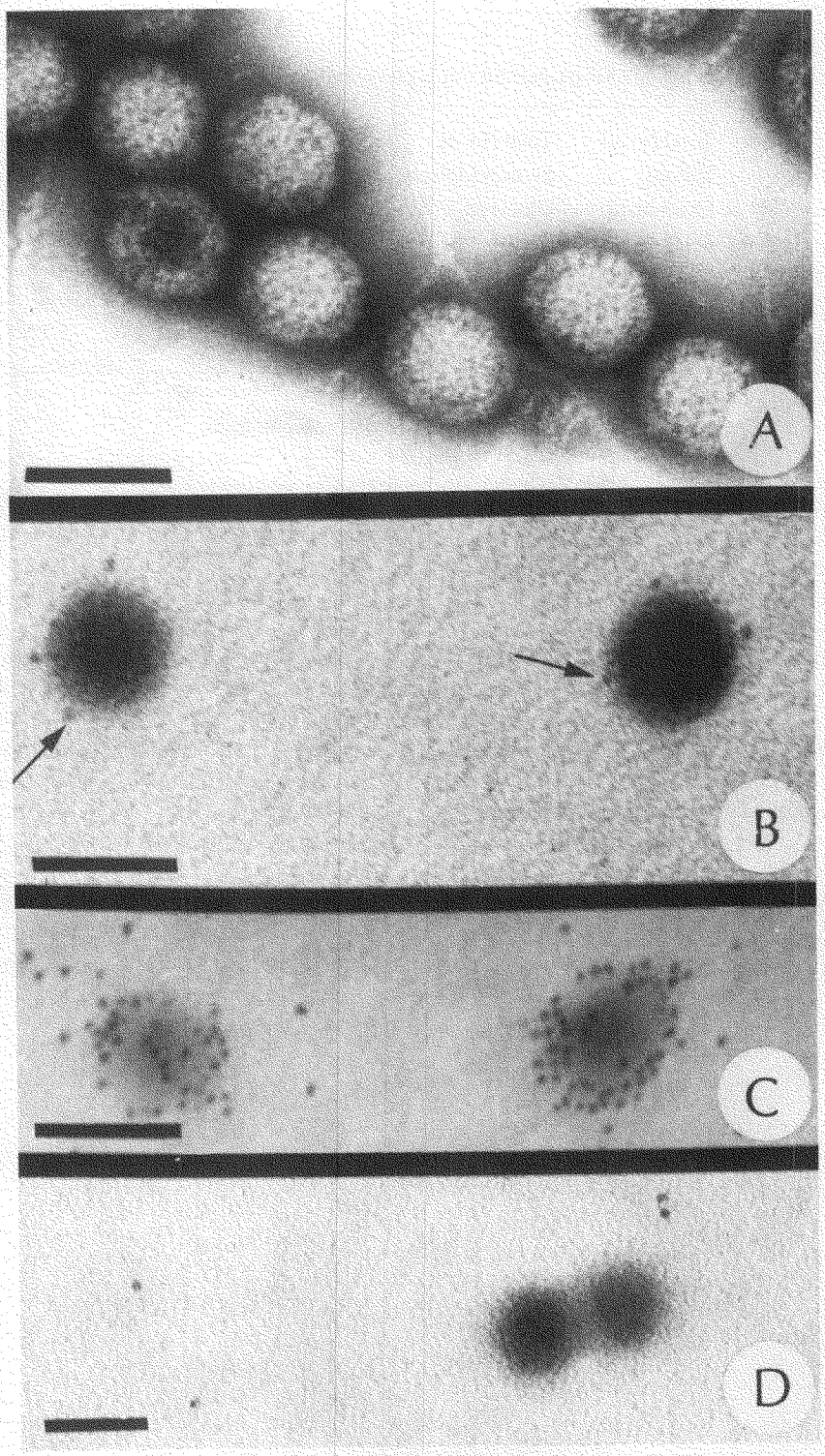

Figure 1. (A) Electron micrograph of reovirus negatively-stained with phosphotungstric acid; (B) Reovirus (unstained) treated with ferritin conjugated to anti-σ1 antibody. Virion-associated ferritin conjugates (arrow) are found as single particles at positions which may correspond to the vertices of the viral icosahedron; (C) Reovirus (unstained) treated with ferritin conjugated to anti-σ3 antibody. Virus-associated ferritin molecules are abundant and randomly distributed. (D) Reovirus (unstained) treated with a heterologous (anti-*C.difficile* toxin-ferritin) conjugate. No virion-associated ferritin molecules are found. In all micrographs, the bar represents 0.2 μm.

Table 1. Association of Anti-σ1 Ferritin Conjugate with Reovirus Particles

	Total No. of Virions Examined	Total No. of Ferritin Molecules Observed	Total No. of Virion-Associated Ferritin Molecules	Average No. of Visible Ferritin Molecules/Virion
[a]Test	50	208	185	3.7
[b]Control	50	35	7	0.14

[a]Test = Virions treated with anti-σ1-ferritin conjugate
[b]Control = Virions treated with anti-*C. difficile* toxin-ferritin conjugate

would in turn result in nonspecific entrapment of conjugates).

To determine the exact location of σ1 on the surface of the virion, virus preparations treated with anti-σ1-ferritin conjugates were thin-sectioned and examined in the electron microscope. The result, shown in Figure 2, suggests that σ1 molecules are indeed localized at the vertices of the viral icosahedrom since these are the positions where most of the electron-dense ferritin particles were found.

We then proceeded to examine reovirus cores to determine whether these locations do represent the tips of the core spikes. Reovirus cores were prepared by treating purified virions with chymotrypsin (2 μg/mL) for 15 minutes at 37°C, followed by centrifugation in a CsCl gradient (Joklik, 1972). Such cores lacked the major outer capsid components, μIC and σ3, but the σ1 protein was found to remain core-associated as confirmed by SDS-PAGE (data not shown). Subsequent electron microscopy (Fig. 3A) showed that the outer viral capsid was indeed removed, whereas the full complement of the icosahedrally-arranged spikes was retained. The cores were then treated with ferritin conjugated to anti-σ1-antibody and prepared for electron microscopy as described above for intact virions. The results are shown in Figure 3B. Since electron-dense stains could not be used for immunoferritin electron microscopy, it was not possible to visualize spikes in these preparations. However, the position and distribution of the core-associated ferritin molecules suggest that the tips of the spikes are probably the sites of recognition for the anti-σ1-ferritin conjugates. This association is also specific since ferritin molecules

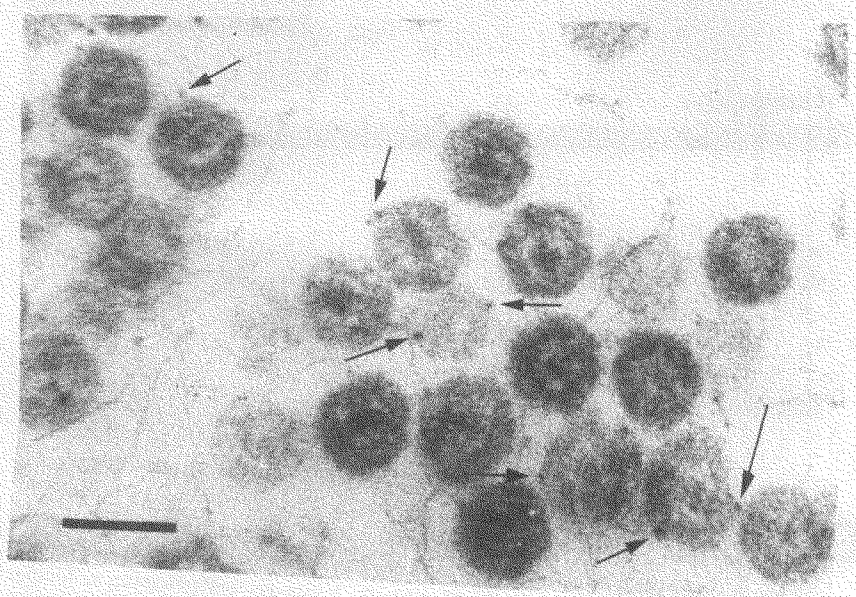

Figure 2. Electron micrograph of thin sections of reovirus particles treated with anti-σ1-ferritin conjugate. Ferritin molecules are found at the vertices of the viral icosahedron (arrows). The bar represents 0.2 μm.

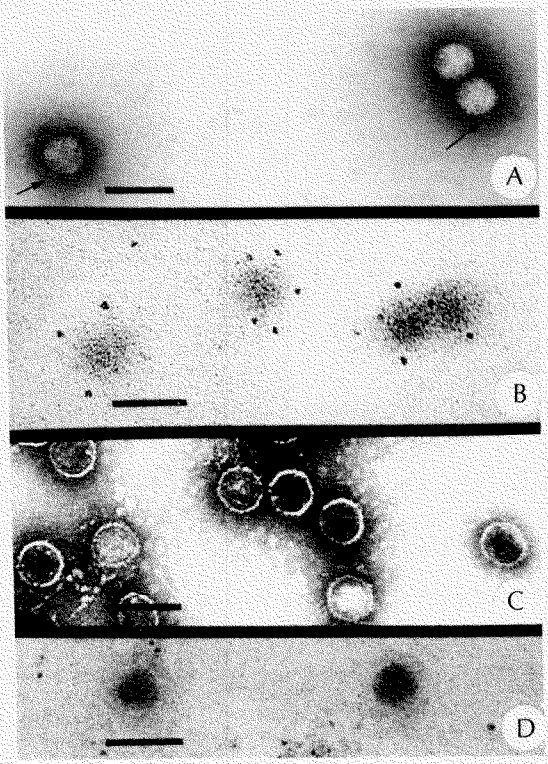

Figure 3. (A) Negatively-stained reovirus cores showing spikes (arrows); (B) Cores (unstained) treated with anti-σ1-ferritin conjugate. Ferritin molecules are found at positions corresponding to the tips of spikes (preparation was unstained to ease visualization of ferritin molecules); (C) Negatively-stained spikeless cores prepared by cold alkali-treatment of cores in (A); (D) Spikeless cores (unstained) treated with anti-σ1-ferritin conjugate. No ferritin molecules are associated with such cores. In all micrographs the bar represents 0.2 μ m.

were not found on cores treated with anti-*C.difficile* toxin-ferritin conjugates (data not shown).

That σ1 is associated with the spikes was further confirmed using cores whose spikes had been removed by cold alkali (0.1 M sodium phosphate buffer, PH 11.8 at 4°C) treatment (White and zweerink, 1976). Figure 3C shows that whereas the spikes were removed completely after such treatment, the remainder of the core remained intact.

When such spikeless cores were treated with anti-σ1-ferritin conjugates and examined under the electron microscope, no core-associated ferritin particles were found (Fig. 3D).

Data from the present immunoferritin electron-microscopic studies therefore strongly suggest that the σ1 protein is located at the vertices of the viral icosahedron, which represent the exposed tips of the core spikes. This is in agreement with the indirect evidence previously

obtained from binding competition studies using various monoclonal antibodies directed against proteins of the reovirus outer capsid [10]. That icosahedral vertices are sites for attachment apparatuses is also evident in the case of adenoviruses [20]. However, it is not known at this time whether most naked, icosahedral viruses use such sites for cellular attachment. The strategic significance of these locations may be accessibility: certainly it is easier to envision cellular receptors interacting with prominent structures rather than with flat surfaces on an icosahedral virus. Such an arrangement may also be important for the subsequent entry of the virus into the cell through multivalent interactions with receptors in the coated pits of the host cell plasma membrane.

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