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Subunit Structure of the Reovirus Spike

S. J. RALPH,[1] J. D. HARVEY,[2] AND A. R. BELLAMY[1]*

Departments of Cell Biology[1] and Physics,[2] University of Auckland, Auckland, New Zealand

Cross-linking reovirus spike protein with the bifunctional reagent dimethyl suberimidate revealed that each spike was composed of a pentameric aggregate of polypeptide λ2.

The double-layered icosahedral reovirus particle contains an inner core which bears on its surface 12 projections or spikes located as if on the 12 fivefold vertices of an icosahedron (8). The core encloses the 10 segments of double-stranded RNA and also contains the various enzymes associated with transcription, capping, and methylation of mRNA (7, 11). The extrusion site for mRNA has not been unequivocally established, but studies with electron microscopy (1) and light scattering (2) indicate that the spike, which is hollow and possesses a 5-nm central channel (8), acts as a conduit for the extrusion of mRNA.

The possible involvement of the spike in the processes associated with the transcription and extrusion of mRNA lends interest to its structure. White and Zweerink (14) demonstrated that the spike is composed entirely of polypeptide λ2. We present here evidence indicating that the spike is a pentamer [$(\lambda 2)_5$] which corresponds to a total molecular weight of 700,000.

Reovirus type 3 (Dearing strain) was propagated and purified as described by Smith et al. (12). ^{125}I-labeled virus was prepared by suspending purified virus in 0.1 M borate buffer (pH 8.5) and reacting it with the Bolton and Hunter reagent (3) obtained from Amersham Corp. Cores were prepared from virus by digestion with 50 μg of α-chymotrypsin per ml, followed by purification as described previously (2). Spike protein was prepared from cores by treatment at pH 11.8 according to the method of White and Zweerink (14). For this purpose, phosphate buffer (0.1 M) at 4°C was adjusted precisely to pH 11.8 on a pH meter calibrated against freshly prepared Sørenson glycine buffer (pH 11.8) (13) also held at 4°C. Cores that had been centrifuged into a pellet were suspended at 4°C in the alkaline phosphate buffer, incubated for 15 min at 4°C, and then recentrifuged (SW50.1 rotor, 30,000 rpm for 45 min at 4°C). The supernatant, which contained the spikes, was held on ice before further treatment.

Reaction of intact virus with the Bolton and Hunter reagent (Fig. 1b) was found to yield virus labeled principally in polypeptides λ2, μ1, μ2, and σ3 (see reference 13 for designation). As anticipated, when this virus was converted to cores by chymotrypsin digestion to remove the outer capsomeres, cores labeled exclusively in the spike protein λ2 were obtained. This observation was of utility since the availability of cores carrying label only in the spike enabled the fate of the spike protein to be monitored by following ^{125}I radioactivity. Treatment of cores at pH 11.8 (Fig. 1a) was found to release polypeptide λ2 but not λ1 or σ2 (Fig. 1a), confirming the observations of White and Zweerink (14). The minor polypeptide λ3 (9, 10) was not released from the core (data not shown).

To estimate the size of spike material released from cores by alkali treatment, ^{125}I-labeled spike protein was analyzed by zone sedimentation on a 5 to 20% glycerol gradient (Fig. 2).

The released spikes sedimented less rapidly than did the phosphorylase A marker (molecular weight, 400,000) but in a manner indicating that the bulk of the spike protein existed as some multimeric unit of polypeptide λ2 (Fig. 2). Therefore, the subunit composition of the spike was investigated further by cross-linking with the bifunctional reagent dimethyl suberimidate according to the method of Davies and Stark (5). When the cross-linking of spikes prepared by alkali treatment was compared with that of standard proteins such as aldolase (4 × 40,000) or phosphorylase A (4 × 100,000), spike protein was found to exhibit a marked tendency to aggregate; cross-linking thus yielded material in which intermolecular rather than intramolecular bands predominated. Consequently, this material would not enter 5% polyacrylamide gels, which were found to be suitable for the resolution of proteins possessing molecular weights in the region of 10^6.

To avoid aggregation and improve the prospects for intramolecular cross-linking, spikes were cross-linked in situ on the core before release by treatment at pH 11.8. For this purpose, 100-μg ^{125}I-labeled cores were suspended at 1 mg/ml in 0.2 M triethanolamine hydrochloride buffer (pH 9.5) and incubated with two successive 0.3-mg additions each of dimethyl suber-

imidate for 30 min at 20°C. Spikes were then released by treatment with alkali, neutralized, boiled in detergent–urea (9, 15) to dissociate the polypeptides, and dialyzed against 0.01 M Tris (pH 8)–0.1% sodium dodecyl sulfate to remove dimethyl suberimidate. The cross-linked peptides were then subjected to electrophoresis on a 5% acrylamide gel (Fig. 3) prepared in the phosphate–urea buffer system (9, 15).

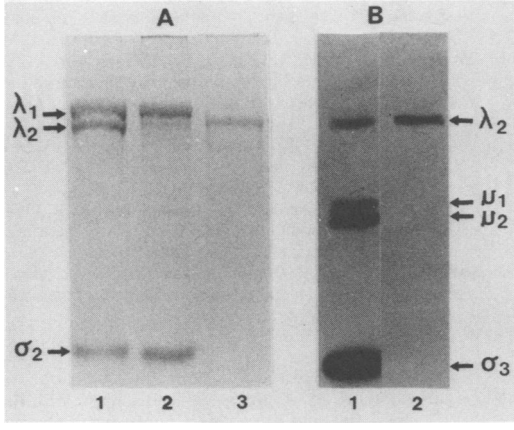

FIG. 1. *(A) Polyacrylamide gel analysis of reovirus cores treated at pH 11.8 by the method of White and Zweerink (14). Cores (channel 1), spikeless cores (channel 2), and spikes (channel 3) were dissociated in detergent–urea and analyzed by electrophoresis on a 10% acrylamide gel in the sodium dodecyl sulfate-phosphate system (9, 12, 15). Stained with Coomassie brilliant blue. (B) Radioautograph of polyacrylamide gel to which sodium dodecyl sulfate-dissociated ^{125}I-labeled virus (channel 1) and cores (channel 2) had been applied.*

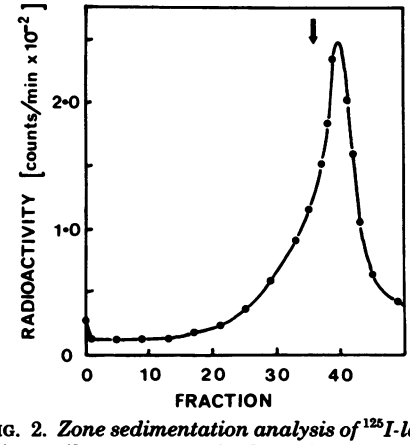

FIG. 2. *Zone sedimentation analysis of ^{125}I-labeled reovirus spikes on a 5 to 20% glycerol gradient (SW27 rotor, 20,000 rpm for 22 h at 4°C). The arrow gives the position of a phosphorylase A marker (molecular weight, 400,000). The direction of sedimentation is from right to left.*

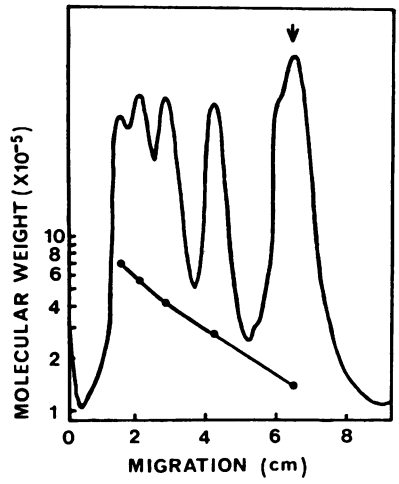

FIG. 3. *Cross-linking of reovirus spike protein. ^{125}I-labeled spikes were cross-linked with dimethyl suberimidate, and the dissociated polypeptides were analyzed on a 5% polyacrylamide gel in the sodium dodecyl sulfate-phosphate system (9, 12, 15). The radioautograph was scanned on a densitometer. The arrow marks the position of the λ2 monomer (molecular weight, 140,000 [12]) which was identified by comparison with non-cross-linked material run on another channel of the same gel.*

Cross-linked spike protein yielded five distinct bands corresponding to the molecular weights predicted for the mono- , di- , tri- , tetra- , and pentameric aggregates of polypeptide λ2 (140,000)n (Smith et al. [12]) (Fig. 3).

The molecular weight of reovirus core protein is 37.3×10^6 (6), and the molecular weight of polypeptide λ2 is 140,000 (12). Since one-third of the core protein is λ2 (12) and there are 12 spikes per core, it may be estimated that there are 7.5 λ2 polypeptides per spike, a value close to the values of 5 or 10 that might be predicted from the location of the spikes on the fivefold axes of the particle. The cross-linking value of five obtained here indicates that the spike is pentameric, whereas the alternative interpretation of 10 subunits formed into two rings of $(\lambda 2)_5$ would imply that cross-linking between adjacent rings has been restricted and requires the unreasonable inference to be made that the spike possesses a frictional drag equivalent to that of a sphere of 70 nm in diameter. The low sedimentation coefficient of the pentameric spike (approximately 10S) may be attributed to the greater frictional drag exerted by this structure when compared with a globular protein of equivalent molecular weight (700,000). A similar observation has been made for the adenovirus type 2 penton base (4) which has a molecular weight of 500,000 and a sedimentation coefficient of 9S. We conclude

that the reovirus spike is pentameric $(\lambda 2)_5$ and that the relatively low sedimentation value observed for this structure reflects the high frictional coefficient associated with its extended hollow cylindrical shape.

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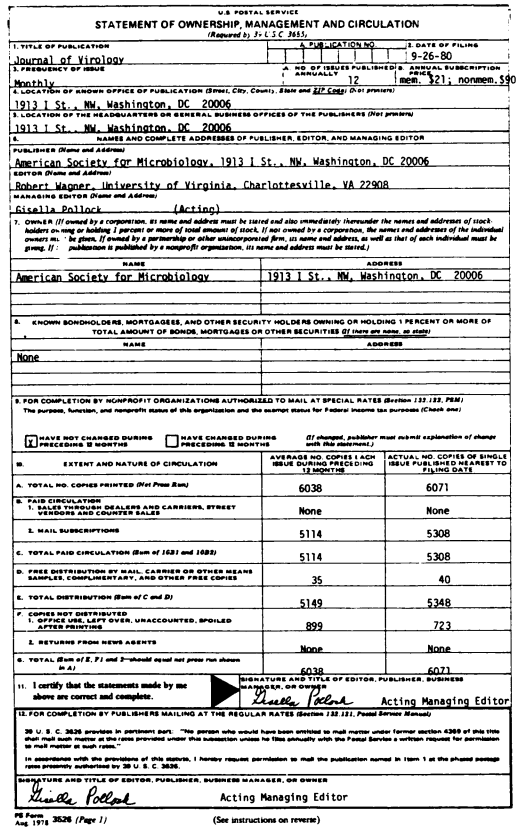

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