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NOTES

Studies on the Topography of Reovirus and Bluetongue Virus Capsid Proteins

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Protein labeling experiments confirm the surface location of proteins 2 and 5 in bluetongue virus, and proteins $\sigma 3$ and $\mu 2$ in reovirus. Lambda 2 is the major surface component of the reovirus core, and proteins 1, 3, and 4 appear to be the outer components of the bluetongue virus subviral particle.

Reovirus and bluetongue virus (BTV) are two animal viruses whose genome consists of 10 segments of double-stranded RNA (12, 16). The reovirion possesses a double-shell capsid arrangement (6, 9, 14). The outer shell consists of proteins $\mu 2$, $\sigma 1$, and $\sigma 3$ (14), and their removal by chymotryptic digestion results in the formation of the core, a particle which comprises the genome RNA and proteins $\lambda 1$, $\lambda 2$, $\mu 1$, and $\sigma 2$ (14), and which possesses RNA dependent-RNA polymerase (transcriptase) activity (13).

Two kinds of BTV particles which differ in their buoyant densities in cesium chloride can be isolated from infected cells. BTV-L (light), the complete virion, has a buoyant density of 1.36 g/cm^3 and contains seven proteins, whereas BTV-D (dense), a subviral structure, has a buoyant density of 1.38 g/cm^3 and contains five proteins (8). Both transcriptase activity and a well defined 32-capsomer structure are evident only in the BTV-D particle. Thus, it seems likely that BTV-D is an inner particle which in turn is surrounded by two proteins in a diffuse layer to form the complete virion, BTV-L (8, 15).

To determine more precisely the vectoral arrangement of the structural proteins of reovirus, BTV, and their subviral particles we performed protein labeling experiments utilizing ^{125}I, dansyl chloride, and fluorescein isothiocyanate (FITC). The rationale was that the reactive residues of proteins situated on the surface of viral particles would be more accessible than the residues of proteins located internally in the particles, and would consequently be labeled to a greater extent. The degree to which each polypeptide species was labeled was determined by dissociating the proteins in urea and sodium dodecyl sulfate (SDS) and subsequently analyzing them by vertical slab SDS-polyacrylamide gel electrophoresis. Proteins labeled with ^{125}I were detected by autoradiography. Qualitative differences in fluorescence of proteins labeled with dansyl chloride or FITC were determined by viewing and photographing the gels under UV illumination. Then the same gels were stained with Coomassie brilliant blue, thus permitting identification of the protein species which exhibited fluorescence.

Figure 1 shows the results of enzymatic iodination (10) of reovirions and reovirus cores. Figure 1A is a densitometric tracing of an SDS-polyacrylamide gel electropherogram of reovirus proteins stained with Coomassie brilliant blue. The pattern of iodination of intact reovirus is shown in Fig. 1B. The species incorporating the greatest amount of label are $\mu 2$ and $\sigma 3$. Core polypeptide $\lambda 2$ is also iodinated, but to a lesser extent, whereas there is very little label in the other polypeptides. Iodination of reovirus cores resulted in significant labeling of $\lambda 2$ with minor appearance of label in $\lambda 1$, $\mu 1$, and $\sigma 2$ (Fig. 1C).

The efficiency with which the major reovirus polypeptides were labeled is shown in Table 1. The polypeptides $\mu 2$ and $\sigma 3$ which form the outer protein layer of reovirions are most extensively labeled. The presence of the outer protein coat limits iodination of $\lambda 2$ as shown by its greater degree of labeling in cores than in virions. The fact that polypeptide $\lambda 2$ is labeled to a greater extent (both in virions and cores)

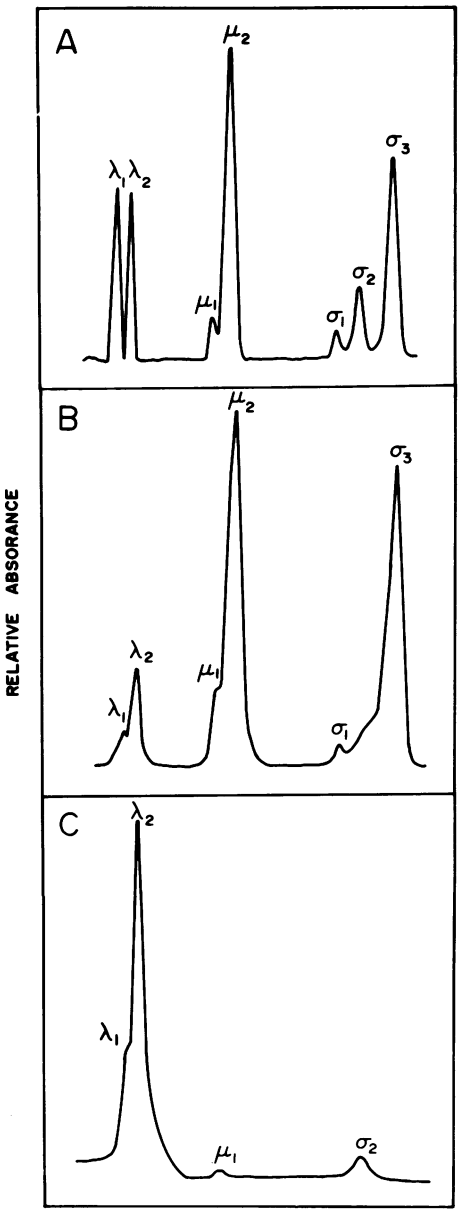

FIG. 1. *Densitometric tracings of SDS-polyacrylamide gels of stained reovirus proteins and of autoradiograms of ^{125}I-labeled reovirus proteins. Enzymatic iodination was performed by incubating 50 μg of viral particles in 100 μliters of 0.1 M phosphate buffer (pH 7.4) with 40 μCi of ^{125}I (New England Nuclear Corp.), 71 ng of lactoperoxidase, and 1 nmol of H_2O_2 at 37 C. After 1 min, 1 nmol of H_2O_2 was again added, and the mixture was incubated for 2 min more (10). The iodinated samples were twice resuspended in phosphate buffer and pelleted at 35,000 rpm for 30 min at 4 C in the SW50 rotor. The resulting pellets were solubilized in 8 M urea, 1% SDS, and 0.1% 2-mercaptoethanol at 100 C for 2 min and then were subjected to electrophoresis for 19 h at 50 mA on 7.5% (bis linked) SDS-polyacrylamide slab gels as previously described (8). Gels were either stained with Coomassie brilliant blue or dried for autoradiography. Densitometric tracings of the stained gels or of the autoradiograms were made in a Gilford spectrophotometer at 562 or 540 nm, respectively. In all panels the direction of electrophoresis was from left to right. (A) Stained gel of reovirus proteins. (B) Autoradiogram of intact reovirus enzymatically labeled with ^{125}I. (C) Autoradiogram of reovirus cores enzymatically labeled with ^{125}I.*

than the other two core proteins λ1 and σ2 suggests that λ2 is more oriented to the surface of the core.

Since iodine reacts only with histidine and tyrosine residues (10), it is possible that the difference between the staining and iodination patterns is due to the fact that not all polypeptides contain the same relative amounts of histidine and tyrosine. However, this is not the case as is shown in Table 1. The relative distribution pattern of ^{125}I in the polypeptides which were denatured prior to chemical iodination is very similar to the distribution pattern of the stained polypeptides. Polypeptide μ2 is iodinated slightly less effective, and σ3 is iodinated slightly more effective.

Figure 2 and Table 2 show the results of enzymatic iodination performed on BTV. A densitometric tracing of a stained SDS-polyacrylamide gel electropherogram of BTV-L proteins is shown in Fig. 2A. The pattern of stained BTV-D proteins is not shown but it is similar to that of BTV-L except that species 2 and 5 are absent.

Figure 2B shows the pattern of iodination of intact BTV-L particles. The greatest incorporation of label was found associated with proteins 2 and 5 which are present in BTV-L only. The major polypeptide 3 was labeled but less than proteins 2 and 5. By contrast, major polypeptide 7 incorporated very little ^{125}I, whereas the minor proteins 1 and 4 incorporated a relatively large amount of label. These results are consistent with our previous suggestion that those proteins present in BTV-L but absent in BTV-D are located on the surface of the BTV-L particle. It seems that proteins 2 and 5 do not entirely mask the surface of the capsomers but are diffusely arranged around them, permitting iodination of residues of proteins in the inner particle. In BTV-D particles the major species labeled are the major protein 3 and the minor proteins 1, 4, and 6 (Fig. 2C). For this reason, these proteins are likely located close to or on the outer surface of BTV-D, whereas polypeptide 7 is located internally in the particle. The relative distribu-

tion of proteins as detected by chemical iodination of denatured BTV-D and L was similar to that detected by staining. Thus, differences in the extent of the iodination of the various BTV proteins are not due to differences in the histidine and tyrosine content.

Agents with different residue specificities were also employed for reovirus and BTV. Preparations of virions, subviral particles, and denatured virus proteins were reacted with FITC at pH 9.6 (11) or with dansyl chloride at pH 8.2 (3). Both reagents primarily react with free amino groups. In all cases, the labeling patterns with FITC were qualitatively similar to those detected by iodination. Somewhat different results were obtained with dansyl chloride. The fluorescence observed in dansylated intact reovirions was associated with polypeptides $\mu 2$ and $\sigma 3$ primarily; polypeptides $\lambda 1$ and $\lambda 2$ were also dansylated but relatively much less than $\mu 2$ and $\sigma 3$. Dansylation of cores showed that all four core proteins exhibited fluorescence which appeared relative to the amount of each species present. These results could be explained by assuming that reovirus cores are permeable to dansyl chloride. One would indeed expect these structures to be permeable to small molecules, since the penetration of nucleoside triphosphates is necessary for the transcriptase activity which is present in

TABLE 1. *Iodination of denatured reovirions, intact virions, and cores*[a]

Protein	Intact virions[b]		Cores[b, c]		Denatured virions[d]	
	Staining	^{125}I (enzymatic iodination)	Staining	^{125}I (enzymatic iodination)	Staining	^{125}I (chemical iodination)
$\lambda 1$	13	2	39	10	10	9
$\lambda 2$	11	6	33	82	10	12
$\mu 1$	2	2	6	2	1	1
$\mu 2$	40	44	—	—	38	31
$\sigma 1$	2	1	—	—	1	1
$\sigma 2$	7	<2	21	5	9	7
$\sigma 3$	24	42	—	—	31	39

[a] Relative amounts of stained or iodinated proteins were expressed as a percentage of the total.
[b] Intact virions and cores were enzymatically iodinated and analyzed on gels as described in Fig. 1.
[c] Proteins $\mu 2$, $\sigma 1$, and $\sigma 3$ are absent from cores.
[d] Reovirions (40 μg) were denatured by boiling for 2 min in 0.1 ml of 8 M urea and 2% SDS and subsequently iodinated in the presence of chloramine T (7). Proteins were precipitated with 10% trichloroacetic acid in the presence of 10^{-3} M NaI, and the pellet was washed several times with 5% trichloroacetic acid (5). The final pellet was solubilized in 8 M urea, 1% SDS, and 1% ME and subjected to electrophoresis on 2 gels as described in Fig. 1. The proteins in one of the gels were visualized by staining, in the other by autoradiography. The slightly different staining pattern in this sample compared to the staining pattern of intact virions is probably due to the fact that not all proteins are precipitated with equal efficiency in trichloroacetic acid.

TABLE 2. *Iodination of intact BTV-L and BTV-D and denatured BTV-L*[a]

Protein	BTV-L[b] (intact)		BTV-D[b, c] (intact)		BTV-L[d] (denatured)	
	Staining	^{125}I (enzymatic iodination)	Staining	^{125}I (enzymatic iodination)	Staining	^{125}I (chemical iodination)
1	2	4	3	21	2	1
2	17	37	—	—	17	16
3	24	24	41	58	20	20
4	1	2	2	8	2	2
5	24	31	—	—	27	31
6	2	1	3	6	3	3
7	29	0	50	6	29	27

[a] Relative amounts of stained and iodinated proteins were expressed as a percentage of the total.
[b] Conditions for iodination and electrophoresis are those described in Fig. 2.
[c] Proteins 2 and 5 are absent in BTV-D.
[d] Conditions for denaturation and subsequent iodination are those described in Table 1.

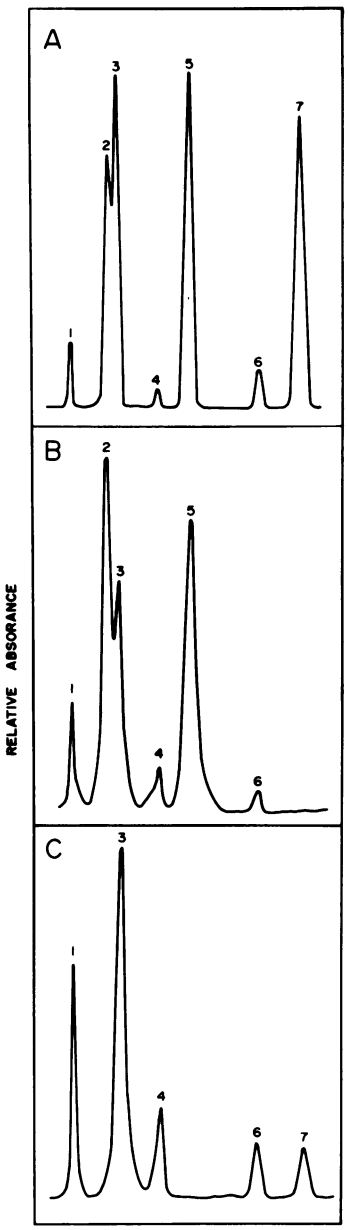

FIG. 2. *Densitometric tracings of SDS-polyacrylamide gels of stained BTV proteins and of autoradiograms of ^{125}I-labeled BTV proteins. All procedures were the same as in Fig. 1. The direction of electrophoresis is from left to right. (A) Stained gel of BTV-L proteins. (B) Autoradiogram of BTV-L particles enzymatically labeled with ^{125}I. (C) Autoradiogram of BTV-D particles enzymatically labeled with ^{125}I.*

these particles. The results obtained with dansylation of BTV-L particles revealed major fluorescence associated with polypeptides 1, 2, 3, and 5 and minor fluorescence with polypeptides 6 and 7. In the case of BTV-D, proteins 1 and 3 exhibited fluorescence of greater intensity than proteins 4, 6, and 7.

In earlier studies on the morphology of BTV (1, 2), this virus was presumed to be quite dissimilar to reovirus; however, there are certain analogous features in their structures. Removal of $\sigma 3$ enhances the visibility of the capsomers of reovirus (6), and the capsomer structure of BTV is revealed by removal of the outer proteins 2 and 5 (8, 15). In BTV the removal of these proteins diminishes its infectivity and activates the particle-associated transcriptase (8, 15). Removal of the outer polypeptides $\mu 2$, $\sigma 1$, and $\sigma 3$ similarly decreases infectivity and activates transcriptase activity in reovirions (4, 13). In subviral structures of both reovirus and BTV there are some structural proteins which are topographically situated on the surface and others which are internal. Whether all of the proteins in the subviral particles participate in the transcriptase activity or whether this activity is mediated only by those proteins located internally (perhaps associated in a ribonucleoprotein complex) remains to be determined.

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