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Sendai Virion Transcriptase Complex: Polypeptide Composition and Inhibition by Virion Envelope Proteins

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Sendai virions, disrupted in 2% Triton X-100 in 1 M KCl, were separated into nucleocapsids and envelope proteins by centrifugation. The nucleocapsids, representing 46% of the virion proteins, had a buoyant density of 1.29 gm/cm^3 in D_2O sucrose. RNA-dependent transcriptase activity associated with them had a ninefold greater specific activity than transcriptase assayed in unfractionated detergent-disrupted virions. These enzyme-active nucleocapsids contained only two polypeptides, the largest virion polypeptide (molecular weight 75,000) and the nucleocapsid structure unit (molecular weight 60,000). Virion envelope proteins, either glycoproteins or nonglycosylated matrix protein, inhibited nucleocapsid-associated polymerase activity; brief heat denaturation abolished their inhibitory activity. Yeast RNA stimulated nucleocapsid-associated enzyme, suggesting that stimulatory polyanions act at the enzyme-template level.

RNA-dependent RNA polymerase (transcriptase) has been described in Sendai virions (8, 13) and in nucleocapsid-like structures (transcriptive complexes) isolated from Sendai virus-infected cells late in infection (12). The enzyme-active structures from infected cells contained two polypeptides. One of these migrated in sodium dodecyl sulfate-polyacrylamide gels like the largest virion polypeptide (molecular weight 75,000). The other migrated like the nucleocapsid structure unit (molecular weight 60,000). These findings suggested that the largest virion polypeptide is involved in transcriptase function. Although structures isolated from virions with properties of nucleocapsids were shown to contain some transcriptase activity (8), their polypeptide composition was not determined. If the largest virion polypeptide is involved in primary transcription of virion RNA, as well as in secondary transcription late in infection, then it must be present in enzyme-active nucleocapsids isolated from virions. In this report, we show that the largest virion polypeptide is indeed present and tightly bound to virion nucleocapsids active in transcription and that the nucleocapsid structure unit is the only other polypeptide resolved in these structures. Inhibitory effects of virion envelope proteins on the nucleocapsid transcriptase and its stimulation by yeast RNA (11) are also described.

MATERIALS AND METHODS

Virus. A Sendai virus clone free of incomplete virions was grown in chicken embryo lung cell cultures at 30 C (13). Radioisotopically labeled virions were prepared as described (12), except labeled precursors were added 16 h after infection and virions were collected 32 h later. Culture medium containing released virus was centrifuged for 10 min at 3,600 × *g* to remove cells and debris. Virus was pelleted (78,000 × *g* at 5 C for 30 min) and suspended in 0.01 M Tris-hydrochloride, 0.03 M NaCl (pH 8.0) for the standard polymerase assay (12) or in 0.01 M sodium phosphate (pH 7.2) for fractionation experiments.

Virus fractionation. The high salt-detergent treatment of Scheid et al. (9, 10) was used with the following modifications. Unlabeled or labeled virions (0.1 to 0.2 mg of protein) were fractionated in 25 ml of 2% Triton X-100 and 1 M KCl. After 20 min at 25 C, the mixture was clarified by centrifugation at 5 C for 20 min at 10,000 × *g*, which removed clumped, incompletely disrupted virions (5% of total protein). The supernatant was centrifuged at 78,000 × *g* for 4 h at 5 C, and the pellet was resuspended in 0.01 M Tris-hydrochloride, 0.03 M NaCl (pH 8.0). The material in the 78,000 × *g* supernatant was further separated into glycopolypeptides and the nonglycosylated envelope polypeptide by dialysis and centrifugation (9, 10).

Isopycnic centrifugation of nucleocapsids. About 2 mg of unlabeled virions, or 0.1 to 0.2 mg of labeled virions, disrupted as described above but in 2.0 ml of Triton X-100 and KCl, were centrifuged at 10,000 × *g* for 20 min at 5 C. The supernatant was layered onto a sucrose gradient column prepared as follows: 2 ml of

1.36 gm/cm³ of D_2O-sucrose was placed in a 1- by 3.5-inch centrifuge tube; a 27-ml linear D_2O-sucrose gradient (1.15 to 1.33 gm/cm³) was formed above it; and a top layer of 2% Triton X-100 and 1 M KCl in D_2O was then added. This preparation was centrifuged at 26,000 rpm for 16 h at 12 C in a Spinco SW 27 rotor. Fractions were collected, and densities were determined (12).

RNA transcriptase assay. Reactions were run as before with [³H]guanosine 5'-triphosphate as the labeled precursor (13), except Triton X-100 was substituted for Triton N-101 when nucleocapsids were tested. Protein determinations were done according to Lowry et al. (6).

Acrylamide gel electrophoresis and radioactivity measurements. These methods have been described in previous reports (12, 13).

Materials. Rabbit hemoglobin was prepared in our laboratory from a lysate of reticulocytes. Bovine serum albumin (BSA) was purchased from Sigma Chemical Co. Fetuin was obtained from Grand Island Biological Company, and ovalbumin was from Worthington Biochemical Corp.

RESULTS

Isolation of enzyme-containing structures from virions. Enzyme-active structures were isolated by centrifugation of Sendai virions disrupted in 1 M KCl containing 2% Triton X-100. Enzyme activity of different virion preparations and 78,000 × *g* pellets derived from them varied, but the latter were consistently fourfold more active than unfractionated virions (Table 1). Incorporation by unfractionated virions and the 78,000 × *g* pellet was linear for 5 h (data not shown). About 46% of virion protein was recovered in the 78,000 × *g* pellet, so there was about a ninefold increase in specific activity of the enzyme (Table 1). The supernatant from the 78,000 × *g* centrifugation contained no polymerase activity (data not shown).

Virions disrupted by detergent-high ionic strength buffer were banded isopycnically in D_2O-sucrose gradients to characterize the rapidly sedimenting material with transcriptase activity. A peak of RNA polymerase activity was found at 1.29 gm/cm³ (Fig. 1), where nucleocapsids band (5, 8), confirming Robinson's (8) observations. Electron microscopy of this material revealed nucleocapsids (not shown).

Polypeptides in the enzyme-containing structure. Enzyme-containing structures were analyzed by acrylamide gel electrophoresis to determine which polypeptides were present. In the following data, we number virion polypeptides according to Mountcastle et al. (7); as before (12) we did not observe polypeptide 4, which is not marked in our figures. Acrylamide gel electrophoresis of the 1.29-gm/cm³ structure revealed three polypeptides (Fig. 2A), the largest virion polypeptide (peak 1), the nucleocapsid structure unit (peak 3), and relatively small amounts of one of the polypeptides in the region of the smaller virion glycopolypeptide (peak 5).

To prevent adventitious binding of irrelevant polypeptides, virions were disrupted in a 10-fold larger volume of buffer (25 ml), which resulted

TABLE 1. *RNA polymerase activity in unfractionated virions and the 78,000 × g pellet from high salt-detergent treated virions*[a]

Experiment[b]	Sample	Counts/min	Counts per min per mg of protein
1	Virions	1,700	45,000
	78,000 × *g* pellet	7,160	400,000
2	Virions	635	28,000
	78,000 × *g* pellet	2,506	245,000

[a] Reactions (13) of 0.1 ml of final volume were incubated for 5 h at 24 C. A complete sample of each was incubated at 4 C for 5 h and counts per minute from these reactions, which ranged from 35 to 60, were subtracted from experimental values.

[b] Each experiment was done with a different preparation of virus.

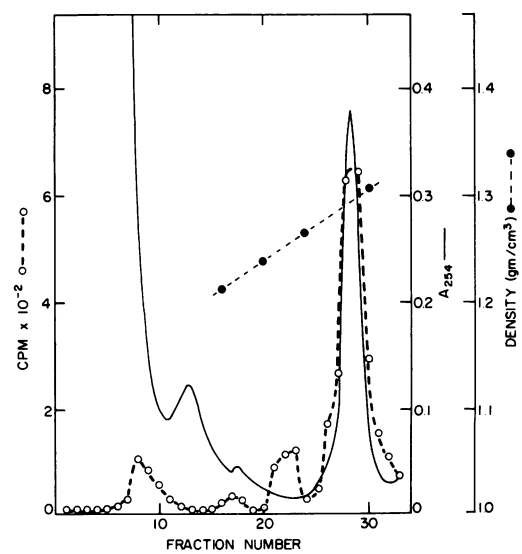

FIG. 1. *Isolation of transcriptase-containing structure from Sendai virions. Virions disrupted in detergent-high ionic strength buffer were centrifuged in a D_2O-sucrose gradient as described in Materials and Methods. Radioactivity represents [³H]guanosine 5'-monophosphate incorporated in a 5-h transcriptase assay.*

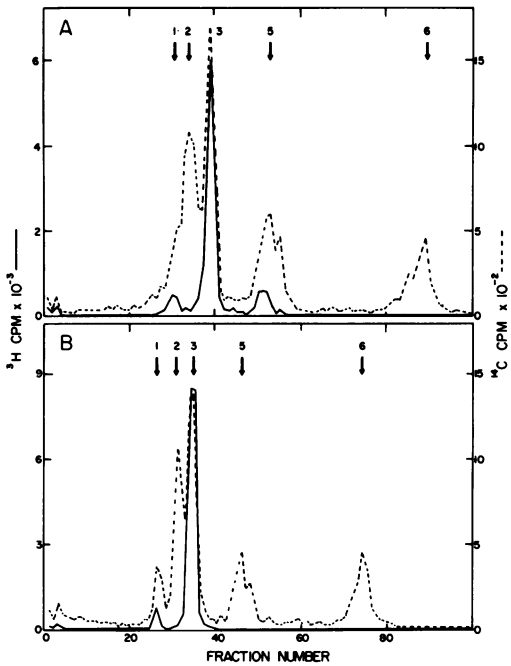

FIG. 2. *Polyacrylamide gel electrophoresis of ^3H-polypeptides in transcriptase-active structures. ^{14}C-polypeptides from Sendai virions were added before electrophoresis. The numbering system follows Mountcastle et al. (7). Migration is from left to right. A, ^3H-polypeptides from the 1.29-gm/cm^3 structure; B, ^3H-polypeptides from the 78,000 × g pellet.*

in the 78,000 × *g* pellet containing only two polypeptides, the largest virion polypeptide and the nucleocapsid structure unit (Fig. 2B). Transcriptase activity was recovered in full from unlabeled virions disrupted at the same concentration. Thus, only two virion polypeptides appear to be involved in transcriptase function.

Inhibition of enzyme activity by viral envelope proteins. As shown above, enzyme-active nucleocapsids contained about half of the total protein of virions, but they had a ninefold greater specific activity than unfractionated virions. This suggests that virion envelope components, perhaps the polypeptides, inhibit polymerase activity. Before testing envelope components for inhibition of polymerase activity, the 78,000 × *g* supernatant was dialyzed to remove KCl and render polypeptide 6 insoluble (10). After centrifugation, the supernatant contained mainly the two virion glycopolypeptides (Fig. 3A), and the precipitate contained mainly polypeptide 6 (Fig. 3B). The peak of radioactivity at fraction 8 in Fig. 3A appears to be an aggregate of virion polypeptides formed during isolation of the envelope fraction, because it is not usually present in unfractionated virions (7, 12) (cf. Fig. 2 and Fig. 3B).

Results in Fig. 4 show that either fraction markedly decreased enzyme activity. Polypeptide 6 inhibited about 80% of polymerase activity when 40 μg was added to a reaction containing 40 μg of nucleocapsids. At the same concentration, glycopolypeptides almost totally abolished enzyme activity. Several experiments summarized in Table 2 were done to investigate whether inhibition by viral envelope proteins was a virus-specific phenomenon. Brief heat denaturation of envelope proteins completely destroyed their ability to inhibit polymerase activity, indicating that protein conformation is important for inhibition. When labeled 50*S* virion RNA was added to a complete reaction containing 400 μg of envelope proteins per ml and the mixture was incubated at 24 C for 16 h, no radioactivity became acid soluble. This indicates that inhibition of transcriptase by envelope proteins is not due to a ribonuclease and confirms earlier evidence against a nuclease activity in Sendai virions (11).

Envelope proteins were isolated from Newcastle disease virions (NDV) by using the same

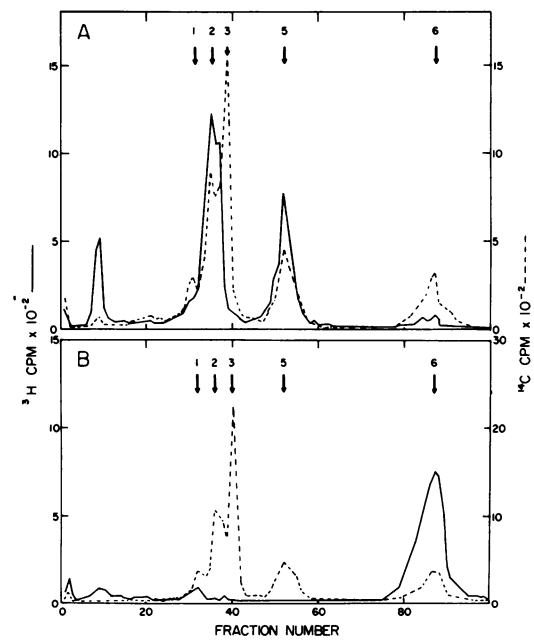

FIG. 3. *Polyacrylamide gel electrophoresis of ^3H-polypeptides from the 78,000 × g supernatant. ^{14}C-polypeptides from virions were added before electrophoresis. A, ^3H-polypeptides which remain soluble after dialysis of the 78,000 × g supernatant, B, insoluble ^3H-polypeptides after dialysis.*

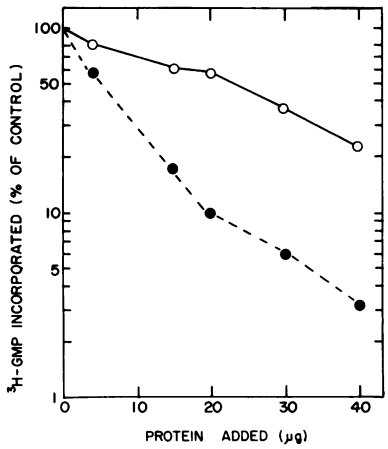

FIG. 4. *Inhibition of transcriptase in nucleocapsids by envelope polypeptides. Radioactivity represents [^{3}H]guanosine 5'-monophosphate incorporated in a 5-h, 0.1-ml transcriptase assay. Counts/min in the control assay were 7,510. Symbols: ○, polypeptide 6; ●, glycopolypeptides 2 and 5.*

TABLE 2. *Inhibition of nucleocapsid-associated polymerase activity*[a]

Additions	Control counts/min[b] (%)
Sendai virus glycoproteins	4
Sendai virus polypeptide 6	23
Heat-inactivated Sendai virus glycoprotein[c]	100
Heat-inactivated Sendai virus polypeptide 6	100
NDV glycoproteins	30
NDV polypeptide 6	59
Ovalbumin	100
Fetuin	100
Hemoglobin	100
BSA	100

[a] Reaction mixtures of 0.1 ml of final volume containing 40 μg of nucleocapsid plus 40 μg of added protein were incubated at 24 C for 5 h. Counts per minute from reactions incubated at 5 C for 5 h were subtracted from experimental values.

[b] Counts/min from reactions with no additions were 5,775.

[c] Virion proteins were heated at 100 C for 2 min.

method as for Sendai virions. They inhibited the polymerase in Sendai viral nucleocapsids, but less efficiently than Sendai viral envelope components. Because any undenatured protein might be capable of inhibiting enzyme activity, several nonviral proteins were tested: 40 μg of either BSA, hemoglobin, or the glycoproteins ovalbumin or fetuin was added to nucleocapsids. None of these proteins inhibited enzyme activity (Table 2).

Stimulation of enzyme activity by yeast RNA. Yeast RNA and other polyanions stimulate Sendai virion transcriptase activity (11), but their mechanism of action is not clear. The results presented above suggested that polyanions might act by reversing inhibitory effects of envelope proteins. If this were true, then yeast RNA should stimulate nucleocapsid-associated enzyme only in the presence of envelope proteins. Results summarized in Table 3 show that yeast RNA stimulates nucleocapsids even in the absence of envelope proteins.

In these experiments, it was noted that some highly active nucleocapsid preparations could not be stimulated at all by yeast RNA, although less active preparations always were stimulated. This indicated that yeast RNA might be reactivating heat-denatured transcriptase. Therefore, a preparation of high activity was partially inactivated by mild heat treatment and tested for stimulation. Data in Table 3 show that yeast RNA restored most of the activity destroyed by heat treatment.

TABLE 3. *Stimulation of virion or nucleocapsid-associated transcriptase by yeast RNA*[a]

Enzyme	Additions	Counts/min	Enhancement over control
Virions	None	1,220	
	30 μg of yeast RNA per ml	1,936	1.6
	300 μg of yeast RNA per ml	2,397	2.0
Nucleocapsids	None	5,020	
	30 μg of yeast RNA per ml	5,937	1.2
	300 μg of yeast RNA per ml	9,179	1.8
Heated nucleocapsids[b]	None	1,384	
	30 μg of yeast RNA per ml	2,521	1.8
	300 μg of yeast RNA per ml	7,591	5.5

[a] Reaction mixtures of 0.1 ml of final volume containing 26 μg of nucleocapsid protein or 39 μg of virion protein and the indicated additions were incubated at 24 C for 5 h. A reaction mixture with nucleocapsids, which was incubated at 4 C, contained 76 counts/min and was subtracted from each experimental value. Background subtraction for virion-containing mixture was 84 counts/min.

[b] Nucleocapsids were incubated at 30 C for 12 min in 0.01 M Tris-hydrochloride, 0.03 M NaCl (pH 8.0). They were then brought to 24 C, mixed with the remaining ingredients of the transcriptase assay (13) and yeast RNA as indicated above, and incubated at 24 C for 5 h.

DISCUSSION

Nucleocapsids isolated from Sendai virions contain an RNA polymerase that is significantly more active than reported previously for unfractionated virions (13). Removal of the virion envelope accounts for the increase in two ways: first, half of the viral proteins are removed, and second, the RNA polymerase is freed from inhibition by envelope proteins. Inhibition by envelope proteins appeared to be specific, requiring undenatured viral proteins.

The enzyme-containing nucleocapsids resemble transcriptive complexes isolated from infected cells (12) in buoyant density, morphology, and polypeptide composition. Thus, similar structures and perhaps the same enzyme are responsible for transcription in virions and in infected cells. More details are needed to determine whether virion polypeptide 1 is indeed identical to "polypeptide 1" detected in cellular transcriptive complexes (12).

The specificity and mechanism of the inhibition of transcriptase by envelope polypeptides may be revealed by further work. Although only viral envelope proteins were inhibitory among those tested, effects of envelope proteins from other viruses as well as additional nonviral proteins need to be examined to learn if the phenomenon is limited to paramyxovirus components. Among possible mechanisms of inhibition, the presence of a protease in the envelope fraction must be considered. However, when various amounts of BSA (up to a 10-fold excess of envelope components) were added either to glycopolypeptide or polypeptide 6 fractions, the inhibition of transcriptase activity was unaffected (data not shown), ruling out gross contamination by a protease.

If the cellular transcriptive complexes are destined for maturation into virions, our data on transcriptase inhibition by envelope polypeptides suggest a mechanism for terminating transcription in the envelopment process. However, if this were true, it is surprising that the virion glycopolypeptides, which are presumed to reside on the outside of the envelope (2), inhibit transcriptase activity. Interaction between nucleocapsid-bound transcriptase and polypeptide 6, which is thought to be on the inside of the envelope (2), seems more likely. Although polypeptide 6 inhibited less efficiently than the glycopolypeptides, this may reflect the poor solubility of polypeptide 6 at the low ionic strength used in the transcriptase assay (13).

Other speculations about enzyme inhibition by envelope polypeptides might be entertained. For example, envelope glycopolypeptides might regulate RNA synthesis at a point in replication before virus maturation.

Recovery of only two virion polypeptides in the enzyme-containing nucleocapsids clearly rules out both glycopolypeptides and polypeptide 6 as part of the transcriptase. Nucleocapsids isolated from virions contained less polypeptide 1 than virions or transcriptive complexes from infected cells. This could result from removal of part of polypeptide 1 by salt-detergent treatment, or it is possible that two virion polypeptides comigrate at this position in the gel (7), one of which is removed during fractionation.

Either the largest virion polypeptide or the nucleocapsid structure unit remains a candidate for the enzyme molecule. Results with vesicular stomatitis virus showed that disruption of virions with high salt and detergent released transcriptase-containing cores that contained the largest virion polypeptide L, a minor component, NS 1, and the major structural polypeptide N (1, 14). Emerson and Wagner (4) showed that L and NS 1, as well as transcriptase activity, could be removed from the core structure by treatment with detergent at sufficiently high ionic strength. When polypeptides L and NS 1 were added back to cores, transcriptase activity was restored, implicating either or both of these polypeptides in transcriptase function. The largest virion polypeptide of Sendai virions is evidently more tightly associated with the nucleocapsid than the L polypeptide of vesicular stomatitis virus, because we have not been able to dislodge it at very high ionic strength (P. Marx and D. W. Kingsbury, unpublished data).

The situation with influenza virus seems less complex. Compans and Caliguiri (3) reported transcriptase-active nucleocapsid structures isolated from influenza virus-infected cells contained only one polypeptide, the nucleocapsid structure unit.

Stimulation of nucleocapsid-associated transcriptase provides further evidence that polyanions act directly on the enzyme. The results suggested that polyanions stimulate by reversing thermal inactivation of the transcriptase.

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