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Poliovirus Polyuridylic Acid Polymerase and RNA Replicase Have the Same Viral Polypeptide

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A poliovirus-specific polyuridylic acid [poly(U)] polymerase that copies a polyadenylic acid template complexed to an oligouridylic acid primer was isolated from the membrane fraction of infected HeLa cells and was found to sediment at 4 to 5S on a linear 5 to 20% glycerol gradient. When the poly(U) polymerase was isolated from cells labeled with [^{35}S]methionine and was analyzed by glycerol gradient centrifugation and polyacrylamide gel electrophoresis, the position of only one viral protein was found to correlate with the location of enzyme activity. This protein had an apparent molecular weight of 62,500 based on its electrophoretic mobility relative to that of several molecular weight standards and was designated p63. When the poly(U) polymerase was isolated from the soluble fraction of a cytoplasmic extract, the activity was found to sediment at about 7S. In this case, however, both p63 and NCVP2 (77,000-dalton precursor of p63) cosedimented with the 7S activity peak. When the 7S polymerase activity was purified by phosphocellulose chromatography, both p63 and NCVP2 were found to co-chromatograph with poly(U) polymerase activity. The poliovirus replicase complexed with its endogenous RNA template was isolated from infected cells labeled with [^{35}S]methionine and was centrifuged through a linear 15 to 30% glycerol gradient. The major viral polypeptide component in a 26S peak of replicase activity was p63, but small amounts of other poliovirus proteins were also present. When the replicase-template complex was treated with RNase T1 before centrifugation, a single peak of activity was found that sedimented at 20S and contained only labeled p63. Thus, p63 was found to be the only viral polypeptide in the replicase bound to its endogenous RNA template, and appears to be active as a poly(U) polymerase either as a monomer protein or as a 7S complex.

The poliovirus single-stranded RNA genome is replicated by an RNA-dependent RNA polymerase (replicase) found in the cytoplasm of infected cells (4). Poliovirion and mRNA syntheses take place in a replication complex that consists of the replicase and a replicative intermediate composed of one complete strand of complementary (negative-strand) RNA and several nascent chains of plus-strand RNA (5, 18). The replicase probably contains one or more virus-coded proteins, but evidence about which viral polypeptide is responsible for replicase activity has been circumstantial. Replicase activity has not been found in extracts of uninfected cells, and poliovirus temperature-sensitive mutants for RNA synthesis have been reported (13). Partial purification of the replication complex indicated that a noncapsid viral protein believed to be NCVP4 was the predominant viral protein component of the complex, but small amounts of other viral and host proteins were also present (9, 23). An RNA-dependent RNA polymerase has been isolated from cells infected with encephalomyocarditis virus but, when the most purified form of the polymerase was analyzed by polyacrylamide gel electrophoresis, at least four virus-coded polypeptides were present, and thus identification of the replicase protein(s) was not possible (24).

We have previously reported the isolation of a soluble poliovirus-specific RNA-dependent RNA polymerase that will copy a polyadenylic acid [poly(A)] template complexed to an oligouridylic acid [oligo(U)] primer (15). The polymerase requires both poly(A) and oligo(U) for activity, is not found until about 2 h postinfection, increases in activity until about 5 h postinfection, and is recovered from the membrane fraction of cells. Because poliovirus infection

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leads to a rapid inhibition of host protein synthesis (22), the above finding suggests that the polyuridylic acid [poly(U)] polymerase is composed of one or more virus-coded proteins. When the poly(U) polymerase was analyzed by glycerol gradient centrifugation, it was found to sediment at 4 to 5S (15). We report here the identification of a virus-specific protein of apparent molecular weight 62,500 (p63) that is associated with this poly(U) polymerase activity. A soluble form of the virus-specific poly(U) polymerase has also been identified that sediments at about 7S and that contains both p63 and the precursor of p63, NCVP2. A protein of the same molecular weight as p63 was the only virus-coded protein in a highly purified form of the endogenous RNA replication complex. This polypeptide therefore appears to be the major viral protein constituent of the poliovirus replicase.

MATERIALS AND METHODS

Extracts of poliovirus-infected cells. Suspension cultures of HeLa cells were infected with poliovirus type 1 as described previously (26) and were treated with 5 μg of actinomycin D per ml at 15 min postinfection. The infected cells (100 ml at 4×10^6/ml) were collected by centrifugation at 3.5 to 5 h postinfection, washed once in Earle saline, suspended in 10 ml of 10 mM Tris-hydrochloride (pH 7.5)–10 mM NaCl–1.5 mM $MgCl_2$, and broken by three cycles of freezing and thawing. When the poly(U) polymerase was isolated, the cells were normally broken with a Dounce homogenizer.

Fractionation of cell extracts. The poly(A)-dependent poly(U) polymerase was isolated as previously described (15) and as outlined in Fig. 1. The endogenous replication complex was isolated by methods similar to those described by Lundquist et al. (23) and as outlined in Fig. 1. A 10-ml volume of cell extract was centrifuged at 2,000 × *g* for 5 min, and the precipitate was suspended in 10 ml of 10 mM Tris-hydrochloride (pH 8.0)–10 mM NaCl (TN buffer) containing 1% (vol/vol) Nonidet P-40 and 0.5% (wt/vol) sodium deoxycholate. The nuclei were removed by centrifugation at 4,000 × *g* for 5 min, and the resulting supernatant was centrifuged at 200,000 × *g* for 1.5 h to sediment the replicase-template complex. This sediment was suspended in 1 ml of TN buffer, 0.25 ml of 10 M LiCl was added, and the solution was allowed to stand overnight at 0°C. When the solution was stored at −20°C, significant losses in replicase activity were observed. The precipitate formed in the 2 M LiCl solution was collected by centrifugation at 12,000 × *g* for 5 min in a Brinkmann microcentrifuge. The pellet was washed once in TN buffer containing 2 M LiCl and then suspended in TN buffer alone. The resuspended LiCl pellet (fraction IV, Fig. 1) was stored at 0°C with little loss in activity during a period of 2 to 3 weeks.

Polymerase assays. The poly(A)-dependent poly(U) polymerase was assayed for 30 min at 30°C as described previously (15). The replicase complexed with its endogenous RNA template was assayed as follows. In a standard reaction, a 50-μl volume of enzyme was assayed in 125 μl of a solution containing: 50 mM *N*-2-hydroxyethyl piperazine-*N'*-2-ethanesulfonic acid (HEPES buffer; pH 8.0); 8 mM magnesium acetate; 8 μM [5,6-^{3}H]UTP (5,500 cpm/pmol); 1 mM each GTP, ATP, and CTP; 4 mM phosphoenolpyruvic acid; pyruvate kinase (3 U/ml); 10 μg of actinomycin D per ml; and 10 mM dithiothreitol. The assay was for 30 min at 37°C. Enzymatic activity was measured by collecting the labeled product on membrane filters (Millipore Corp., 0.45 μm) after precipitation with 7% trichloroacetic acid (0.33 saturated with sodium pyrophosphate) in the presence of 100 to 200 μg of added carrier RNA. The filters were dissolved in 5 ml of Bray scintillation fluid (New England Nuclear Corp.) and then counted after the addition of 10 ml of Aquasol-2 scintillation fluid (New England Nuclear Corp.). Adding the Aquasol-2 increased the efficiency of counting about twofold. Whenever ^{35}S-labeled proteins were present during polymerase assays, the acid-precipitable ^{35}S counts, in addition to normal background radioactivity, were subtracted.

Polyacrylamide gel electrophoresis. Proteins were precipitated with acetone, using 5 μg of cytochrome *c* as the carrier protein. Residual acetone was removed by evaporation, in vacuo, and the precipitate was dissolved in 20 μl of electrophoresis sample buffer (20) and heated at 95 to 100°C for 3 min. Samples were analyzed by electrophoresis on 12.5% polyacrylamide slab gels in the presence of sodium dodecyl sulfate ($NaDodSO_4$). The procedure was as described by Laemmli (20), except that the running gel buffer was 1.5 mM Tris-hydrochloride (pH 8.0), the stacking gel buffer was 0.5 M Tris-hydrochloride (pH 6.8), and the electrode buffer was prepared with 6 g of Tris base, 28.8 g of glycine, and 1 g of $NaDodSO_4$ per liter of buffer. The gels were run at 150 V (constant voltage) for about 3.5 h, fixed, stained with Coomassie brilliant blue, and destained in the presence of a mixed-bed resin (AG501-X8 D, Bio-Rad Laboratories). To increase the sensitivity of detection, some gels were equilibrated with a 2,5-diphenyloxazole (PPO)–dimethyl sulfoxide solution by the procedure of Bonnar and Laskey (6). The gels were dried and autoradiographed at −70°C, using Kodak XR-5 film. The molecular weight standards used in this study were bovine serum albumin (68,500), ovalbumin (45,000), vesicular stomatitis virus N protein (52,500), and vesicular stomatitis virus G protein synthesized in vitro (63,500) (19).

Glycerol gradient centrifugation. Because sucrose precipitated from solutions when acetone was added to precipitate proteins, glycerol was used to form the gradients used in this study. Samples of 0.5 to 0.7 ml each were centrifuged through 10.8-ml gradients, using an SW41 rotor (Spinco) at 4°C, as described in the figure legends. When gradients were collected from the top, a collection device from Hoefer Scientific Instruments was used; 50% glycerol was pumped through a hypodermic needle into the bottom of the centrifuge tube.

Phosphocellulose chromatography. Phosphocellulose was prepared by the method of Burgess (7). The resin was equilibrated with 50 mM Tris (pH 8.0), and a column (0.8 by 6 cm) was prepared. The column

was then equilibrated and washed with 50 ml of 50 mM Tris (pH 8.0)-20% glycerol-0.5% Nonidet P-40-2 mM dithiothreitol-10 μg of ovalbumin per ml (TGND-OA). The polymerase sample in TN buffer-20% glycerol (12 ml) was mixed with 12 ml of TGND-OA buffer and applied to the column at a flow rate of 0.2 ml/min. The column was washed with 20 ml of TGND-OA buffer and then eluted with a 60-ml linear gradient of 0 to 1 M KCl in TGND-OA. Fractions of 0.9 ml each were collected and stored at 4°C.

Materials. [5,6-^{3}H]UTP was obtained from New England Nuclear Corp. in a 50% ethanol solution. To remove the ethanol, the solution was reduced to 0.1 its original volume with a Buchler Evapomix fraction evaporator. ^{35}S-labeled poliovirions were purified by differential centrifugation and by banding the virus in a CsCl density gradient (12). Vesicular stomatitis virus proteins labeled with [^{35}S]methionine were synthesized in a cell extract in vitro (19). The sources for other materials were as previously described.

RESULTS

To compare the viral protein components of the poly(U) polymerase and the endogenous replicase, a crude fractionation was first achieved. In each fraction, total endogenous activity and poly(U) polymerase activity were separately measured (Table 1). The membrane-bound components of the infected cells were extracted with detergents and separated into components soluble or precipitated in 2 M LiCl (Fig. 1). The fraction with the majority of the endogenous activity (fraction IV, Fig. 1) was recovered by detergent extraction of a "nuclear" fraction (large membrane vesicles) and by precipitation in 2 M LiCl (23). The majority of the membrane-bound poly(U) polymerase was recovered in fraction I (Fig. 1) by detergent extraction of a small-membrane-vesicle fraction that was soluble after 2 M LiCl treatment (15). Although not shown in Table 1, a significant amount of poly(U) polymerase activity was also recovered directly as a soluble protein in a 20,000 × *g* (or 200,000 × *g*) supernatant of the cytoplasmic extract (fraction VI, Fig. 1).

Poly(U) polymerase protein. To analyze the polypeptide composition of the poly(U) polymerase, the detergent-solubilized activity (fraction I) was sedimented through a glycerol gradient. As reported earlier (15), it sedimented at 4 to 5S at the approximate position of bovine serum albumin (molecular weight, 68,500) (Fig. 2). If the infected cells were labeled with [^{35}S]methionine and each glycerol gradient fraction was analyzed by polyacrylamide gel electrophoresis, a single viral protein was found to correlate with the location of enzyme activity (Fig. 2). This polypeptide had a molecular weight of about 62,500 compared with those of a variety of standards and was one of the labeled protein bands that was found in cytoplasmic extracts of poliovirus-infected cells (lanes A and B, Fig. 2). Although other viral polypeptides were also present in the gradient, most were known virion constituents (lane C, Fig. 2), and none was distributed selectively in the enzymatically active fractions. It therefore appears that the poly(U) polymerase solubilized from cellular membranes consists of a single viral polypeptide, which we will denote p63.

When the poly(U) polymerase activity in the soluble portion of the cytoplasm (fraction VI, Fig. 1) was analyzed by glycerol gradient centrifugation, it was found to sediment significantly faster than the membrane-associated polymerase extracted with detergent and 2 M LiCl (Fig. 3). The soluble enzyme sedimented at about 7S when compared with a bovine serum albumin sedimentation marker run in a parallel gradient. Analysis of the viral proteins sedimenting with the 7S polymerase by polyacryl-

TABLE 1. *RNA polymerase activity in fractions from poliovirus-infected cells*[a]

Cell fraction assayed	Total endogenous replicase (pmol of NTP incorporated)	Total poly(U) polymerase (pmol of UTP incorporated)
I	60	2,550
II	580	64
III	<0.1	552
IV	1,304	448
V	<0.1	1,840

[a] Poliovirus-infected cells (8 × 10^8) were collected at 3.5 h, broken by freezing and thawing, and fractionated as indicated in Fig. 1. Polymerase activity was measured, using [^{3}H]UTP as the labeled substrate, and the values for endogenous replicase activity represent the calculated picomoles of all four ribonucleoside triphosphates incorporated.

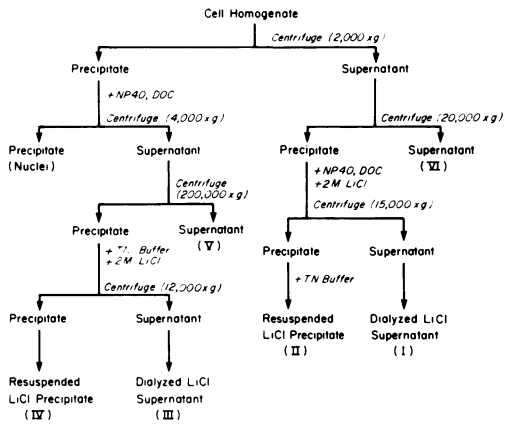

FIG. 1. *Fractionation of poliovirus-infected cells. Fractions designated by roman numerals were assayed as described in Table 1. NP40, Nonidet P-40; DOC, deoxycholate.*

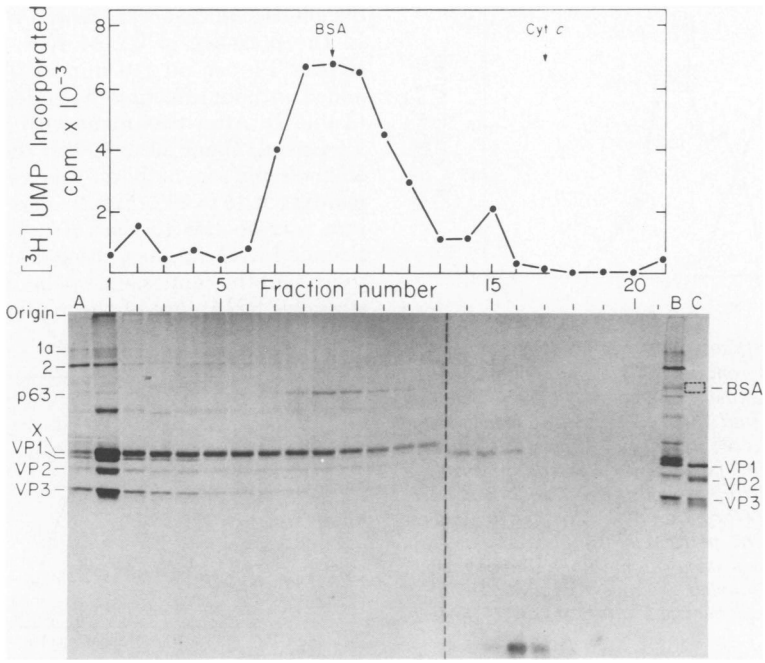

FIG. 2. *Glycerol gradient centrifugation (top) and polyacrylamide gel electrophoresis (bottom) of membrane-associated poly(U) polymerase. Cell fraction I was isolated from cells labeled with [^{35}S]methionine (2.5 to 4 h) and was centrifuged on a 5 to 20% glycerol gradient at 35,000 rpm for 39.5 h at 4°C. The gradient was collected in 0.5-ml fractions from the bottom, and part of each fraction was assayed for poly(U) polymerase activity. The proteins in the remaining portion of each fraction were analyzed by NaDodSO$_4$-polyacrylamide gel electrophoresis. Fractions 1 through 13 were analyzed on the gel shown at the left, and fractions 14 through 21 were analyzed on the gel shown at the right. The direction of centrifugation was from right to left. ^{125}I-labeled bovine serum albumin and cytochrome c (Cyt c) were used as sedimentation markers and were analyzed in a parallel gradient. Lanes A and B contained ^{35}S-labeled cytoplasmic poliovirus proteins; and lane 3 contained ^{35}S-labeled poliovirion proteins and a bovine serum albumin marker that was located by staining the gel.*

amide gel electrophoresis revealed that p63 and NCVP2 (the precursor of p63) cosedimented with polymerase activity (Fig. 4). This was confirmed by cutting out and counting the labeled protein bands from each lane in the gel. The amounts of radioactivity recovered in p63 and NCVP2 were maximal in the same fraction that had maximal polymerase activity (fraction 12, Fig. 4) (data not shown). Approximately three times as much radioactivity was recovered in NCVP2 as in p63 in the peak fraction of polymerase activity. This ratio increased on the leading side of the peak (5.0 in fraction 17, Fig. 4) and decreased on the trailing side of the peak (1.4 in fraction 8, Fig. 4). The bands for NCVP2 in lanes 8 to 12 (Fig. 4) were distorted by unlabeled host proteins migrating at about the same position as NCVP2. The distribution of the other viral proteins in the gradient was not found to correlate with enzyme activity.

The 7S polymerase activity from the glycerol gradient was purified further by gradient elution from phosphocellulose. A single peak of polymerase activity was found eluting from the column at about 0.14 M KCl (Fig. 5). Polyacrylamide gel electrophoresis revealed that both p63 and NCVP2 co-chromatographed with the poly(U) polymerase activity (Fig. 5). About 3.5 times as much radioactivity was recovered in NCVP2 as in p63. The p63 band in fraction 56 was diffused by a large unlabeled protein band running in approximately the same position in the gel. The two other major viral proteins appeared to elute slightly ahead of the polymerase activity peak.

Therefore, p63 was found to cosediment with both the 4S polymerase activity and the 7S polymerase activity and to co-chromatograph with the polymerase activity on phosphocellulose. NCVP2 was also found to cosediment with the 7S polymerase and to co-chromatograph with the polymerase on phosphocellulose. The p63 component alone appears to be active as a poly(U) polymerase, but the activity of NCVP2 alone is uncertain. Although a significant amount of NCVP2, was recovered in fraction 1 (Fig. 2), little poly(U) polymerase activity was

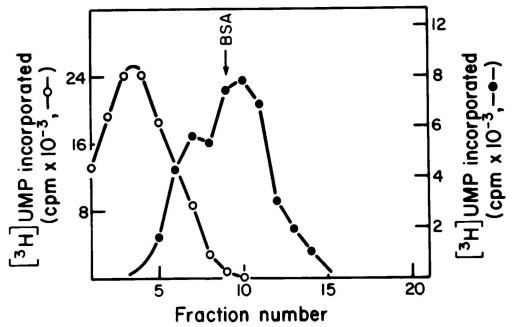

FIG. 3. *Centrifugation of the poly(U) polymerase activity isolated from the soluble and membrane fractions of the cytoplasm. The soluble enzyme (fraction VI, Fig. 1)* (○) *and the detergent-extracted enzyme (fraction I, Fig. 1)* (●) *were isolated from cells infected for 4.5 h. A 0.5-ml portion of each was analyzed in parallel by centrifugation through 5 to 20% glycerol gradients at 25,000 rpm for 40 h. The gradients were collected from the bottom in 0.5-ml fractions, and each fraction was assayed for poly(U) polymerase activity. An ^{125}I-labeled bovine serum albumin (BSA) sedimentation marker was analyzed in a parallel gradient.*

detected. Further investigation is required to establish whether or not NCVP2 alone has polymerase activity.

Viral proteins of endogenous replicase. To define the protein of the endogenous replicase, virus-specific proteins were labeled with [^{35}S]methionine from 1 h, 50 min to 3 h, 30 min postinfection, and the fraction enriched in endogenous activity (fraction IV, Table 1) was prepared. This fraction (lane B, Fig. 6) contained most of the labeled proteins found in cytoplasmic extracts (lanes A and C, Fig. 6). When this fraction was centrifuged through a 15 to 30% glycerol gradient, a peak of endogenous replicase activity was found sedimenting at about 26S along with a heterogeneous distribution of more rapidly sedimenting activity (Fig. 6). One labeled polypeptide distributed in the gradient like the endogenous activity; it has a molecular weight of 62,500. Traces of other labeled polypeptides (mainly VP1 and VP3, lane D, Fig. 6) were also evident but were not selectively distributed in the 26S region.

The broad distribution of enzyme activity in Fig. 6 could be a consequence of binding of the enzyme to replicative-intermediate RNAs, molecules that are known to have a broad sedimentation distribution (3). Because of the possibility that nuclease could remove the portions of the nascent plus strands that were not hybridized to negative strands—and thus could help purify the endogenous replicase—the fraction with endogenous replicase was treated with RNase T1. In the presence of 0.2 M KCl, up to 2 μg of RNase T1 per ml (10 min at 37°C) could be added without affecting the endogenous activity (Table 2). After treatment with 2 μg of RNAse T1 per ml, about 30 to 40% of the input endogenous replicase activity was recovered, sedimenting at 18 to 20S (Fig. 7). This sedimentation rate corresponds to that of poliovirus double-stranded RNA (2), suggesting that after nuclease treatment the replicase stays bound to a double-stranded RNA core of the replicative interme-

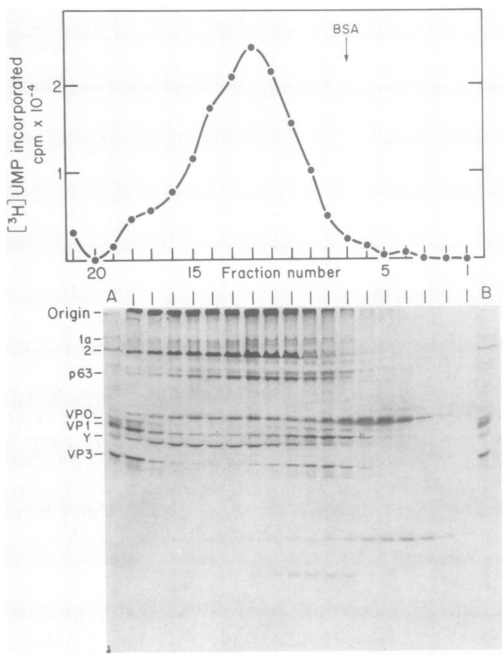

FIG. 4. *Analysis of the viral proteins associated with the 7S poly(U) polymerase by polyacrylamide gel electrophoresis. (Top) Fraction VI was isolated from cells labeled with [^{35}S]methionine (2.75 to 4.75 h) by centrifugation of the cytoplasm at 200,000 × g for 1 h. A 0.7-ml portion of the high-speed supernatant was sedimented through a 5 to 20% glycerol gradient at 38,000 rpm for 22 h at 4°C. The gradient was collected in 0.5-ml fractions from the top, and each fraction was assayed for polymerase activity. (Bottom) The protein composition of each fraction was analyzed by NaDodSO$_4$-polyacrylamide gel electrophoresis. Lanes A and B contained labeled cytoplasmic poliovirus proteins. Because virions would not be present in the 200,000 × g supernatant, VP2 (found only in mature virions) should not be present. We therefore assume that the band between VP1 and VP3 is band Y, a protein that previously was found in cells infected with poliovirus-defective interfering particles and that migrates in approximately the same position as VP2 (11). BSA, Bovine serum albumin.*

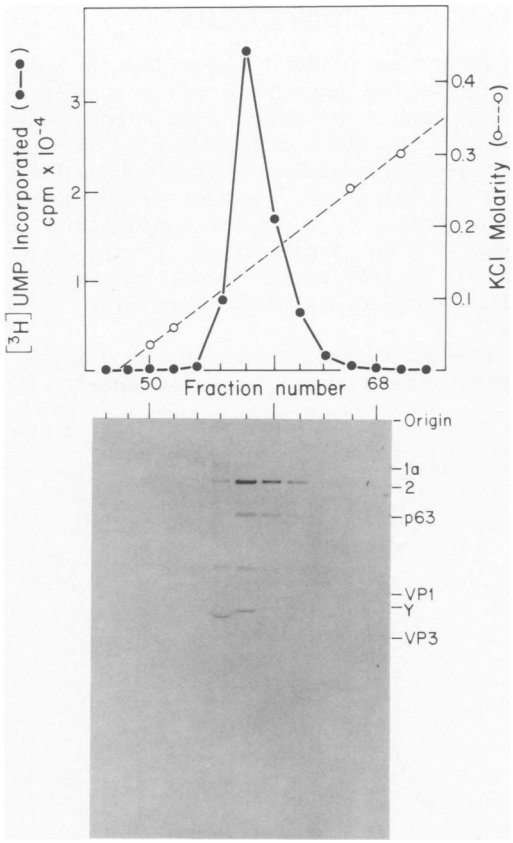

FIG. 5. *Phosphocellulose chromatography (top) and polyacrylamide gel electrophoresis (bottom) of the 7S poly(U) polymerase. The peak fractions of polymerase activity from a glycerol gradient similar to the one in Fig. 4 were pooled and applied to a phosphocellulose column. The column was washed and eluted with a linear salt gradient (0 to 1 M KCl) as described in the text. Every second fraction was analyzed for poly(U) polymerase activity. The labeled proteins in the fractions containing polymerase activity were analyzed by polyacrylamide gel electrophoresis. No activity was found in the fractions eluted from the column at the higher salt concentrations.*

diate and can add nucleotides at gaps in the structure.

When the 18–20S form was prepared from [^{35}S]methionine-labeled cells, a polypeptide of molecular weight 62,500 (p63) was the only viral polypeptide recovered in the 18 to 20S region (Fig. 7). The X-ray film used for the autoradiogram shown in Fig. 7 was sensitive to a level of radioactivity about 10 times less than the amount of radioactivity present in p63 in fractions 7 and 8 (Fig. 7). If other viral polypeptides were present in the 18 to 20S region, then they contained less than 10% of the radioactivity present in p63. The molecular weight of p63 was determined by analyzing a variety of molecular-weight standards (lanes A and B, Fig. 7) and by making a semilogarithmic plot of their molecular weights versus the distance of migration in the gel. This plot gave a molecular weight of 62,500 for the replicase protein and 77,000 for NCVP2 in lane C, Fig. 7. Butterworth (8) previously reported a molecular weight of 77,000 for NCVP2 in type 2 poliovirus, and Abraham and Cooper (1) reported a value of 79,000 for NCVP2 in type 1 poliovirus. Because p63 was the only viral protein recovered in the 20S peak, it appears that p63 is the endogenous replicase protein. The exact size correspondence of the endogenous replicase protein (Fig. 7) and the poly(U) polymerase protein on polyacrylamide gels (Fig. 2) suggests that the same protein, p63, is responsible for both activities.

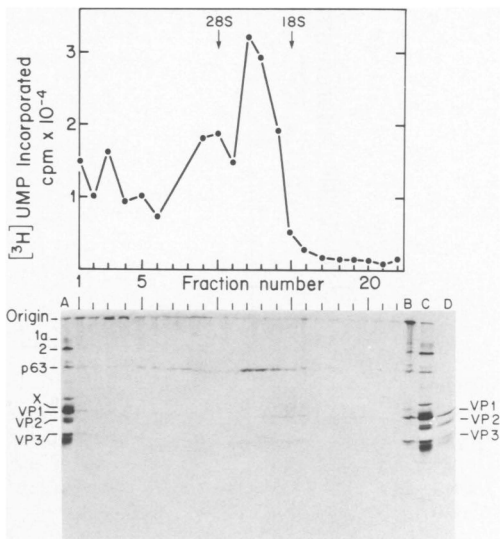

FIG. 6. *Glycerol gradient centrifugation (top) and polyacrylamide gel electrophoresis (bottom) of the endogenous replicase-template complex. Cell fraction IV (Fig. 1) was isolated from infected cells labeled with [^{35}S]methionine, layered on a 15 to 30% linear glycerol gradient, and centrifuged in an SW41 rotor at 25,000 rpm for 19 h at 4°C. The gradient was collected in 0.5-ml fractions from the bottom. The direction of centrifugation was from right to left. HeLa cell rRNA sedimentation markers were run in a parallel gradient. A portion of each fraction was assayed for endogenous replicase activity, and the labeled proteins in the remainder of each fraction were analyzed by NaDodSO$_4$-polyacrylamide gel electrophoresis as described in the text. Lanes A and C contained ^{35}S-labeled poliovirus proteins from an infected cytoplasmic extract; lane B contained a portion of cell fraction IV that was analyzed on this gradient; and lane D contained ^{35}S-labeled poliovirion proteins.*

TABLE 2. *Effect of RNase T1 digestion on endogenous replicase activity*[a]

RNase T1 (μg/ml)	Replicase activity ([^{3}H]UMP incorporated, cpm × 10^{-3})
0.0	8.9
0.2	9.0
2.0	9.9
20.0	3.3

[a] Cell fraction IV (5 μl; Fig. 1) was incubated with the indicated amount of RNase T1 in 50 mM HEPES (pH 8.0)–33 mM $MgCl_2$–0.2 M KCl (10-μl total volume) for 10 min at 37°C. Endogenous replicase activity was then measured for 10 min at 37°C by adding 115 μl of the standard assay reaction mixture (see text).

DISCUSSION

From these results it appears that the poliovirus-specified polypeptide p63 is a required component of the soluble poly(U) polymerase and the template-bound endogenous replicase. The poly(U) polymerase appears to be active in both a 4S form and 7S form of the enzyme and is dependent on a poly(A) template and an oligo(U) primer for its activity (15); the endogenous replicase mainly copies the negative-strand template to which it is very tightly bound (17).

Previous studies have indicated that one polypeptide predominated in fractions enriched for

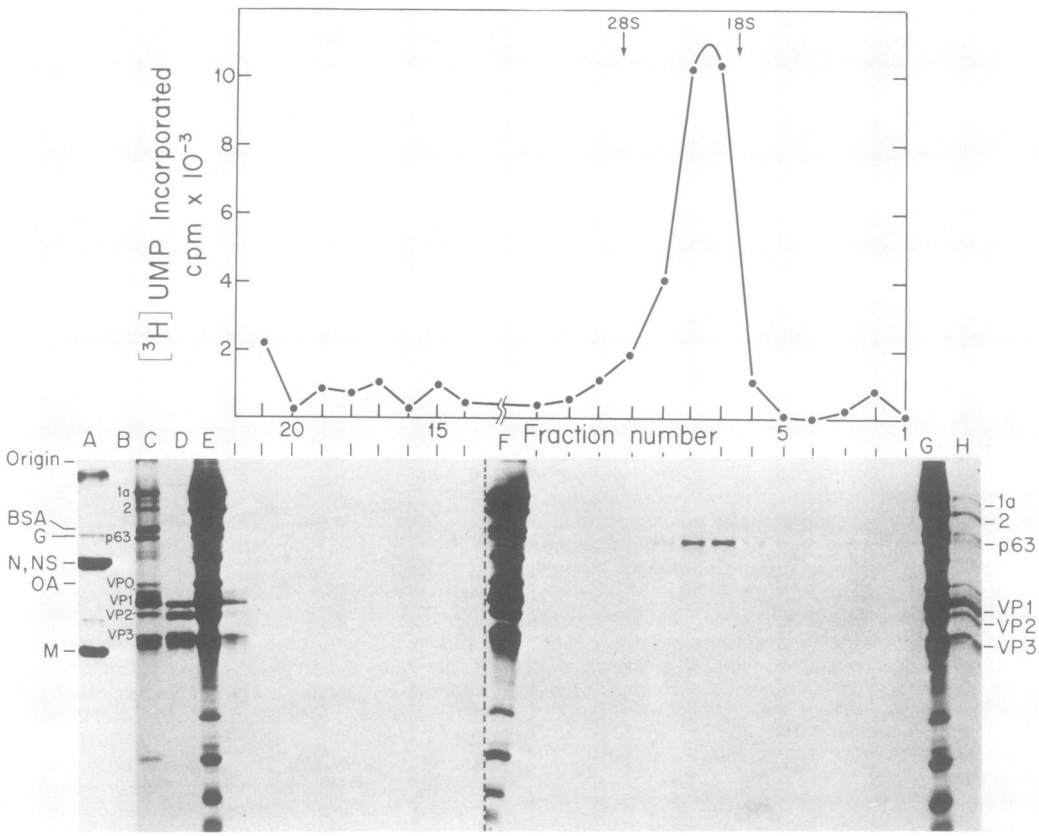

FIG. 7. *Glycerol gradient centrifugation (top) and polyacrylamide gel electrophoresis (bottom) of the RNase T1-treated endogenous replicase-template complex. The procedures were as described in the legend to Fig. 6, except that cell fraction IV was treated with 2 μg of RNase T1 per ml as described in Table 2, footnote a. The gradient contained 0.2 M KCl and was centrifuged at 40,000 rpm for 6 h at 4°C. It was collected from the top as described in the text, and the direction of centrifugation was from right to left. Fractions 1 through 13 were analyzed on the gel shown at the right, and fractions 14 through 22 were analyzed on the gel shown at the left. Lane A contained labeled vesicular stomatitis virus proteins (G, N, NS, M) synthesized in vitro (19); lane B contained unlabeled bovine serum albumin (BSA) and ovalbumin (OA) (detected by staining the gel with Coomassie brilliant blue; positions are shown in the left margin); lane C contained a portion of fraction IV before RNase T1 treatment; lane D contained ^{35}S-labeled poliovirion proteins; lanes E, F, G, and H contained ^{35}S-labeled cytoplasmic poliovirus proteins.*

endogenous replicase activity (9, 23). By digestion with RNase T1, a purification of the endogenous replicase to a single viral protein was achieved here. The polypeptide we designate as p63 appears from its mobility relative to that of NCVP2 to be the same polypeptide designated in various publications as NCVP3B (14, 25), NCVP4 (9, 23), and p58 (1).

Although p63 is the only viral protein in the endogenous replicase, there may be host cell proteins involved also. Thus far, we have not obtained sufficient protein representing the endogenous replicase to identify or exclude host components. Even if p63 acts alone during elongation, there could well be other proteins needed for initiation. In fact, it has been suggested that the protein found covalently linked to the 5′ end of poliovirion RNA (designated VPg) acts as a protein primer for initiating viral RNA synthesis (16, 21).

The polioviral genome encodes (from 5′ to 3′) three major protein products: NCVP1A, the capsid precursor; NCVPX, a 35,000-dalton polypeptide; and NCVP2, a 77,000-dalton polypeptide (13). Genetic studies have implied that the replicase and capsid precursor are at opposite ends of the genome (13). Furthermore, much of NCVP1A can be deleted without reducing replicase function (11). These results strongly suggest that p63 must be derived from NCVP2 (and in turn from its short-lived precursor, NCVP1.5, or 1B). In confirmation of this suggestion, V. Ambros and D. Baltimore, using the technique of Cleveland et al. (10), have shown that the partial protease digestion products of p63 are present among those of NCVP2 (unpublished data).

The poly(U) polymerase that was extracted from the membrane fraction of infected cells with detergent and 2 M LiCl appears to be active as a 4S monomer of p63. The polymerase from the soluble portion of the cytoplasm, however, sediments at about 7S and may be a complex of p63 and NCVP2 because both proteins cosediment and co-chromatograph with the activity on phosphocellulose. The molecular weight implied by the 7S sedimentation rate is consistent with this proposal, but the ratios of radioactivity found in NCVP2 and p63 suggest that a 1:1 complex of p63 plus NCVP2 is not the only structure present unless NCVP2 contains much more methionine than p63. A dimer of NCVP2 may also be present, and this would explain why about three times as much radioactivity was recovered in NCVP2 as in p63. A dimer of p63 is a third possibility. Further purification and characterization will be necessary to clarify these relationships.

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