Purification and Properties of a Host Cell Protein Required for Poliovirus Replication in Vitro*

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A host cell protein required for poliovirus RNA-dependent RNA replicase activity in vitro has been purified several thousand-fold from uninfected HeLa cell postmitochondrial supernatant. A single protein of apparent M, = ~67,000 daltons and pl 6.3 is associated with this "host factor" activity. Poly(U)-Sepharose chromatography of the template-dependent replicase isolated from poliovirus-infected cells results in the complete loss of replicase activity if a salt gradient is used to develop the column. Host factor elutes early in the salt gradient and restores replicase activity to protein fractions eluted later in the gradient. The host factor, estimated to be present at 50,000–100,000 copies/cell, interacts physically with replicase.

Virions of poliovirus contain a single-stranded RNA molecule of positive polarity which is replicated by a virus-specific polymerase activity (1). Early attempts to isolate and purify the poliovirus RNA replicase were complicated by the difficulty of releasing the enzyme from its tight association with cellular membranes and with its endogenous template. A template-dependent form of the enzyme was finally isolated (2) by taking advantage of a simple and sensitive assay for a poly(A)-oligo(U)-directed poly(U) polymerase activity (3) which co-purifies with the viral polypeptide believed to be responsible for replicase activity in poliovirus-infected cells (4). Poly(U) polymerase and template-dependent RNA replicase co-purify through gradient elution from phosphocellulose and step elution from poly(U)-Sepharose (2) and are both associated with the viral protein p63 (5).

Analysis of the poliovirus replication system follows earlier studies on the replication of RNA bacteriophages (6). That replication system involves a number of host cell proteins which are commandeered by a single phage-encoded replicase protein (7–12). Active replicase is a tetramer which comprises the one phage protein plus three host proteins whose normal function is in the translational machinery of the cell (12, 13).

A fourth cellular protein, host factor (14, 15), is not a subunit of the replicase complex per se. Like the other host-encoded polypeptides, host factor appears to play a role in the initiation of phase RNA synthesis, but its function in the uninfected cell is still a mystery. All four of the host cell proteins are normally found in association with ribosomes and are released by incubation of these structures in high salt.

Dasgupta et al. (16) found that gradient elution of the poliovirus replicase from a column of poly(U)-Sepharose was accompanied by the complete loss of replicase activity. By analogy with the phage system, they prepared a high salt extract of ribosomes from uninfected HeLa cells and showed that it was capable of reconstituting replicase activity. The function of the host cell protein fraction, termed "host factor," could be provided by the addition of oligo(U) to the gradient-eluted replicase protein, suggesting a role for the host factor in initiation of RNA synthesis. None of the known initiation or elongation factors for protein synthesis showed any evidence of host factor activity. Involvement of one or more host cell proteins in the replication of encephalomyocarditis virus has also been reported (17).

In the process of preparing host factor for further studies on poliovirus replication in vitro, we have found that approximately 80% of the host factor activity is actually in the soluble fraction of homogenized cells. We have succeeded in purifying the host factor several thousand-fold from uninfected HeLa cell cytoplasm and have characterized some of the physicochemical properties of this protein. When the phosphocellulose-purified, primer-independent preparation of poliovirus replicase is chromatographed on a poly(U)-Sepharose column, host factor elutes in the salt gradient ahead of poly(U) polymerase activity. If these early fractions are added back to fractions containing poly(U) polymerase from the same column, template-dependent replicase activity is completely reconstituted. This observation strongly suggests that crude preparations of replicase are contaminated with this host protein and that the host factor dependence is not simply an artifact of the gradient elution. The host factor can be shown to interact physically with replicase. Some of the properties of the host factor-stimulated replicase reaction are discussed.

**EXPERIMENTAL PROCEDURES**

Materials. Radiochemicals were purchased from New England Nuclear or from Amersham Corp. [U-14C]-labeled nucleotides were obtained from P-L Biochemicals, poly(A) from Miles Laboratories, oligonucleotides from Collaborative Research, phosphocellulose PC-11 from Whatman, and DEAE-Sephadex, Sepharose 4B, Sephacryl S-200 Superfine, Sephadex G-25, poly(U)-Sepharose 4B, and chromatofocusing reagents from Pharmacia Fine Chemicals. Proteins used as standards for molecular weight determinations were obtained from Boeringer-Mannheim, or Sigma. Osmium tetroxide was from Pierce and phenylmethylsulfonyl fluoride was from Calbiochem.

* Portions of this paper (including "Experimental Procedures," part of "Results," and Figs. 1 and 2) are presented in miniprint as prepared by the authors. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 82M-231, cite the authors, and include a check or money order for $4.00 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

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Poly(U)-dependent polymerase activity was assayed by incubation of 10 pg (0.2-0.5 nmol) to a 30 pmol reaction mixture containing a final concentration of 50 mM Hepes-KOH, pH 7.5, 50 mM NaCl, 0.1 mM dithiothreitol, 10 mM magnesium acetate, 5 pmol of poly(U), and either 6 pmol oligo(U) or the indicated amount of host factor. The reaction was allowed to proceed for 30-60 min (30°C), acid-precipitable radioactivity was measured as previously described (2), except that filters were dried under a heat lamp for 30-60 min before the addition of Bray’s cocktail.

Preparation of poly(U)-Sepharose. Poly(U)-Sepharose was isolated and purified as previously described (16) using immobilized oligo(U) to gel electrophoresis under denaturing conditions (21). All buffer solutions used in this procedure were treated with 0.1% diethyl pyrocarbonate and autoclaved, and glass centrifuge tubes were treated with PDS and then autoclaved.

Preparation of oligo(U) for use in replication assay. Oligo(U) (1 mg/ml in 10 mM Tris-HCl, pH 8.3) was dialyzed against 50 mM NaCl, 0.1 mM dithiothreitol, and 10% ethylene glycol (v/v) against Buffer II (0.2-0.5 mg/ml) to a 50-ml reaction mixture containing 30 pmol poly(U) polymerase to be assayed for host factor activity. The solution was then washed with 3 volumes of Buffer II containing 0.2 M NaCl and 10% ethylene glycol (v/v) and 0.5 mM EDTA.

Sodium dodecyl sulfate-polycrylamide gel electrophoresis. Polymerase fractions were analyzed by electrophoresis through 10% polyacrylamide/0.13% sodium dodecyl sulfate gels exactly as described by Davis et al. (18).

Sedimentation velocity. Sedimentation coefficients were measured by sedimentation velocity using a Spinco model E ultracentrifuge in a 10-mm 50 ml analytical cell at 20°C and a 50,000 rpm cell at 15°C. The solutions were examined at wavelengths of 254 and 550 nm. Values for sedimentation coefficients were taken from Refs. 22-23.

Oxygen-breathing activity of Sepharose 6B and coupling to poly(U) polymerase. Sepharose 6B was activated according to the procedure of Dancer et al. (20). Poly(U)-Sepharose 6B (0.2-0.3 mg/ml) in coupling buffer (0.1 M NaHCO3, pH 8.4, 0.5 M NaCl) was applied to a 50 ml coupling column of the Sepharose 6B. The resin was washed with 3 volumes of 0.2 M glycine-HCl pH 2.7 and coupling buffer. Resins

A abbreviations used are: PMSF, phenylmethylsulfonyl fluoride; HRPEPS, 4-(2-hydroxyethyl)-1-piperazinethanesulfonic acid; Tris, tris(hydroxymethyl)methane; MES, 2-(N-morpholino)ethanesulfonic acid; PIPES, 1,4-piperazine-diethanesulfonic acid.
for control experiments (see text) were prepared by coupling of ovalbumin in place of poly(U) polymerase or by direct reaction of activated Sepharose with ethanalamine, without prior coupling with protein.

affinity chromatography of host factor. Poly(U) polymerase-Sepharose (16) was prepared by coupling buffer (30 ml distilled water, 5 mM di-thiophosphate, 3.5 mM Mg-acetate) to the matrix at a flow rate of about 4 ml/hr. An equal volume of buffer was collected and re-applied to the column. This procedure was repeated 3 times and then the column was washed with 3 volumes of binding buffer containing 0.1 M KCl and 1 ml di-thiophosphate (200 mM) and magnesium acetate was added to 3.5 mM immediately before chromatography. The protein was applied to the affinity column at a flow rate of about 0.1 ml/hr. An equal volume of buffer was collected and re-applied to the column. This procedure was repeated 3 times and then the column was washed with 3 volumes of binding buffer containing first 0.1 M KCl and next 0.3 M KCl. A portion of each fraction was assayed for host factor activity.

RESULTS

Purification of host factor.

(i) Fractions I and II: crude extract and high speed supernatant. Nela cells (5.5 x 10^9) were allowed to lyse in 80 ml of Buffer I and were homogenized as described under Experimental Procedures. The cell suspension was centrifuged at 29,000 x g for 30 min and the resulting supernatant was taken as fraction I. A second centrifugation step (10,000 x g) yielded fraction II, the postnuclear supernatant or S10.

(ii) Fraction III: ammonium sulfate precipitation. Approximately 76-80% of the host factor activity was found between 40-60% saturated ammonium sulfate. Precipitated protein was resuspended in and dialyzed against Buffer A.

(iii) Fraction IV: DEAE-Sephacel chromatography. Fraction III host factor was applied to a column of DEAE-Sephacel equilibrated with Buffer A (24) in a linear 50-450 mM KCl gradient (Figure I). The peak fractions of active host factor (eluting between 80-120 mM KCl) were pooled and could be stored at -20°C for at least 6 months without detection of activity. Activity was concentrated at the bottom of the pool (usually about one-third, or the equivalent of 1.6 x 10^9 cells) was subjected to a chromatofocusing step.

(iv) Fraction V: Chromatofocusing. The host step in our purification scheme exploits a column chromatographic method, chromatofocusing, which separates proteins according to their isoelectric points. In preparation for the final fractionation step, a portion (10.9 ml) of the fractions as described above was applied to a column of Sephadex G-25 in Buffer B and was then applied to a column (0.5 x 18 cm) of Polybuffer Exchanger 94. Polybuffer 74-45, pH 4.0 was used to develop the gradient. The eluted solution of Polybuffer did not interfere with the host factor activity determinations (data not shown). Host factor eluted in a single peak centered around pH 6.25 (Figure 2). The active material was dialyzed (1°C) versus Buffer A containing 50% glycerol.

The chromatofocusing step provides an estimate of the isoelectric point of the host factor. However, the measured pH of elution is probably slightly lower than the true pH because of the effects of an electrostatic field between the surface of the exchanger pores and the external buffer, similar to a Donnan potential (29,30).

(v) Host factor stability. The 50% glycerol dialysis step serves to concentrate the protein as well as to maintain its stability on storage. Fraction V (usually 50 ml glycerol) was stored at -20°C for at least 3 months and will withstand several cycles of freezing and thawing.

The effects of pH, buffer composition, and salt concentration on the stability of host factor activity have also been examined. Host factor is stable between pH 6.3 and 7.0 in the presence of buffers containing TRIS, PIPES, MES, and potassium phosphate. However, the replica case itself is exquisitely sensitive to phosphate concentrations above approximately 0.2 M. These conditions are shown in the absence of KCl are reproducible almost 100% of the activity was lost after 7 days; this loss was prevented if 50 mM KCl was included in the storage buffer.

(vi) Removal of Polybuffer. Fraction V host factor is stable in the presence of Polybuffer and is adequate for most purposes. However, dialysis on a chromatograph on a macrodiemetric agarose, exploiting the apparent presence of one or more free amino sulfhydryl groups on the host factor molecule, can be used to remove Polybuffer. Activity is quantitatively recovered by elution with Buffer A/50% glycerol/10 mM di-thiophosphate.

Physicochemical properties of host factor.

(1) Molecular weight estimation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. As discussed earlier, polyacrylamide gel electrophoresis of Fraction V host factor revealed the presence of two bands of protein detectable by Coomassie blue staining. The more slowly migrating protein elutes exactly with the peak of host factor activity obtained on chromatofocusing (vide supra) and its corresponding molecular weight is about 69,000 daltons.

(2) Gel filtration. The molecular weight of host factor was estimated under non-denaturing conditions by gel filtration through a column of Sephacyl S-200 Superose, as described under Experimental Procedures. The column was calibrated with 8 typical globular proteins; partition coefficients were calculated against the corresponding logarithms of molecular weight or diffusion coefficient. From these calibration curves, host factor "homomer" has a molecular weight of about 67,000 daltons and the "heterodimer" has a molecular weight of about 135,000 daltons.

Values for the Stokes radius of the standard proteins were treated as described by Siggel and Mischo (26). The Stokes radius of host factor was estimated at 32 A.

(3) Sedimentation velocity. Native proteins were run in parallel glycerol gradients (15-30%) and the sedimentation coefficient of the native host factor protein (s20w) was obtained using the formula of Martin and Ames (33).

The methods used to calculate the molecular weight of host factor are valid only for globular proteins. We therefore estimated the frictional ratio (f/fn) from the Stokes radius and the molecular weight determined by sedimentation velocity (33). Because the amino acid composition of host factor has not yet been determined, it was not possible to calculate a value for the partial specific volume (0.32). If host factor is a typical protein of N = 73,5 ml/mg, the frictional ratio is then 1.06. A completely well-behaved, globular protein would have an ideal frictional ratio of 1.0.

Cellular Location of Host Factor—Host factor activity was first identified in a protein fraction isolated from a HeLa cell ribosomal salt wash (16). This activity could not be positively associated with purified preparations of any of the known initiation or elongation factors involved in protein synthesis (16). In addition, poliovirus replicase, the activity of which is dependent upon the presence of host factor, is itself soluble (2, 36). As a first step in trying to understand better how host factor functions in poliovirus replication, we undertook a careful study of the subcellular localization of this protein.

Host factor activity was identified using an assay which measures restoration of template-dependent RNA polymerase activity (37) to highly purified preparations of p63; this protein demonstrates only poly(U) polymerase activity in the absence of host factor. Uninfected HeLa cells were lysed in a hypotonic buffer and, after homogenization, nuclei and membranes were removed by low speed centrifugation. High speed centrifugation (130,000 x g) yielded a soluble fraction (S130) and a ribosomal pellet from which a 0.5 M KCl wash was prepared. The ribosomal salt wash and the high speed supernatant (S130) were chromatographed on DEAE-Sephacel in 0.15 M KCl; under these conditions, host factor activity was recovered in the flow-through fractions, while cellular RNAs were retained by the resin. This step was required to eliminate contaminating RNA which interfered with the assay. The results of five separate fractionations are summarized in Table I: from 63-92% of the total host factor activity was recovered in the soluble fraction of the cell. When the high speed centrifugation was preceded by addition of extra salt to 0.2 M, the yield of host factor activity in the S130 fraction was increased (Table I, Experiments 4 and 5); however, the specific activity of the resulting fraction was lower, and in designing the purification scheme which is presented below, we have not included the additional salt.

Purification of Host Factor—The results of a purification

![Figure 1](https://via.placeholder.com/150)
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HeLa cells (4.5 × 10⁶) were collected by centrifugation, washed once with phosphate-buffered saline, and resuspended in 80 ml of Buffer I. After incubation for 10 min at 0 °C, the cells were disrupted by Dounce homogenization, and the lysate was subjected to centrifugation for 30 min at 27,000 × g (4 °C). The resulting supernatant was then centrifuged at 130,000 × g for 2 h to yield a ribosomal pellet and a soluble fraction (S130). The ribosomes were resuspended in Buffer A containing 0.5 M KCl and incubated at 0 °C for 45 min, and the high salt wash was recovered in the supernatant after centrifugation at 130,000 × g for 2 h. The S130 fraction and ribosomal salt wash were dialyzed versus Buffer A containing 0.15 M KCl and applied to a column (1.0 × 8 cm and 0.7 × 8 cm, respectively) of DEAE-Sephacel. Host factor activity was not retained under these conditions. The flow-through fractions were pooled and assayed for activity. In Experiment 5 (conducted in parallel with Experiment 4), KCl was added to the S27 to give 0.2 M above the initial concentration.

Table I

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Total host factor activity in S130</th>
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<tr>
<td></td>
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<tr>
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<td>2</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>63</td>
</tr>
<tr>
<td>4</td>
<td>82</td>
</tr>
<tr>
<td>5 (+ 0.2 M KCl)</td>
<td>92</td>
</tr>
</tbody>
</table>

Table II

Purification of host factor

HeLa cells (4.5 × 10⁶) were collected by centrifugation, washed once with phosphate-buffered saline, and resuspended in 80 ml of Buffer I. After incubation for 10 min at 0 °C, the cells were disrupted by Dounce homogenization, and the lysate was subjected to centrifugation for 30 min at 27,000 × g (4 °C). The resulting supernatant was then centrifuged at 130,000 × g for 2 h to yield a ribosomal pellet and a soluble fraction (S130). The ribosomes were resuspended in Buffer A containing 0.5 M KCl and incubated at 0 °C for 45 min, and the high salt wash was recovered in the supernatant after centrifugation at 130,000 × g for 2 h. The S130 fraction and ribosomal salt wash were dialyzed versus Buffer A containing 0.15 M KCl and applied to a column (1.0 × 8 cm and 0.7 × 8 cm, respectively) of DEAE-Sephacel. Host factor activity was not retained under these conditions. The flow-through fractions were pooled and assayed for activity. In Experiment 5 (conducted in parallel with Experiment 4), KCl was added to the S27 to give 0.2 M above the initial concentration.

Figure 2. Chromatofocusing of host factor. Fraction IV host factor (approximately 1 mg) was dialyzed versus 25 mM imidazole-Cl pH 7.4, applied to a column (0.5 × 18 cm) of Polybuffer Exchanger 94, and eluted with Polybuffer 7A pH 4.0. Fractions (0.5 ml) were collected, the pH (0) of every fifth fraction was measured, and 1 μl portions of the indicated fractions were assayed for host factor activity (B).

Figure 3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of various host factor fractions. Lane 1 contains the following protein standards: phosphorylase b (94,000 daltons); bovine serum albumin (67,000 daltons); ovalbumin (45,000 daltons); carbonic anhydrase (30,000 daltons). Lanes 2, 3, and 4 contain, respectively, Fraction II (10 μl), Fraction III (3 μl), and Fraction IV (30 μl) host factor. Fraction V host factor (125 μl) was first precipitated with 10 volumes of acetone at −20 °C in the presence of 6 μg of cytochrome c, resuspended in 30 μl of sample buffer, and then denatured and subjected to electrophoresis (Lane 5) through a 10% gel in parallel with the other samples.

of host factor from 4.5 × 10⁶ cells are summarized in Table II. Details are provided in Miniprint.

Purity of Fraction V Host Factor—The scheme outlined above yields host factor which has been purified several thousand-fold. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to monitor the purity of the host factor preparation (Fig. 3). Upon comparison of Fractions II,
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III, and IV (Fig. 3, Lanes 2, 3, and 4) with Fraction V host factor (Lane 5) it is evident that a striking purification has been achieved. Two bands can be detected by staining: a more slowly migrating band found slightly behind the bovine serum albumin marker (in some preparations, this band appears as a doublet of two very closely spaced bands) and a faster moving band, migrating almost coincident with carbonic anhydrase. (The protein at the bottom of Lane 5 is a contaminant in the carrier protein used during acetone precipitation.) A separate experiment, in which a portion of each gradient fraction from the chromatofocusing column was concentrated by precipitation with acetone and then analyzed by gel electrophoresis, revealed that the higher molecular weight protein alone co-eluted exactly with host factor activity; the smaller protein eluted somewhat later in the gradient (data not shown).

If approximately half of the Fraction V protein is represented in the higher molecular weight band(s), and if this band does in fact correspond to host factor, it can be estimated that host factor is present to the extent of 50,000-100,000 molecules/cell.

Chromatographic Separation of Host Factor and Replicase—Phosphocellulose-purified poliovirus replicase (containing primer-independent activity) was chromatographed by gradient elution from a column of poly(U)-Sepharose 4B (Fig. 4), and different regions of the gradient were examined for the presence of host factor activity. The peak fractions of poly(U) polymerase activity were combined as Pool II, and fractions flanking this peak were combined as Pools I and III (Fig. 4). Poly(U) polymerase eluted between 0.1-0.2 M KCl in the gradient and contained no replicase activity (Table III). Host factor activity eluted early in the gradient: when protein in Pool I was added back to poly(U) polymerase from the same column (Pool II), replicase activity was reconstituted. Pool I itself contained no replicase activity, and addition of Fraction IV host factor (which is fully active when assayed in the presence of Pool II—see Table III) was without effect. Pool III contained neither replicase nor host factor activity, either alone or in combination with Pools I or II or Fraction IV host factor. Therefore, the activity present in Pool I must originally have been present in the phosphocellulose replicase and was separated away from poly(U) polymerase during the second chromatographic step.

Affinity Chromatography of Host Factor on Replica-Sepharose—To investigate the possibility that host factor interacts with the poliovirus replicase protein, the replicase was purified from 1.6 x 10⁹ poliovirus-infected HeLa cells and was covalently coupled to Sepharose as described under “Experimental Procedures.” The resin was equilibrated with binding buffer, the composition of which was chosen to conform as closely as possible to that of the replicase assay buffer. Fraction IV host factor (about 3 mg or the equivalent of 4 x 10⁹ cells’ worth of host factor) was dialyzed into 50 mM HEPES-KOH, pH 8, 4 mM dithioerythritol, 20% glycerol. Magnesium acetate was added to 3.5 mM and the protein was then applied to the affinity column and eluted as described under “Experimental Procedures.” No host factor activity was detectable in the flow-through fractions of the column. Approximately 50-55% of the activity was eluted with 0.1 M KCl and 45-50% was eluted with 0.3 M KCl. Less than 5% of the total protein (27) recovered was contained in the 0.3 M KCl fraction.

To ensure that the interaction of Fraction IV host factor with the affinity medium was specific, control experiments were performed using columns of Sepharose 4B alone, Sepharose 4B coupled only with ethanolamine, and Sepharose 4B coupled with ovalbumin and then with ethanolamine. From 80-85% of the host factor activity was recovered in the flow-through fractions in these determinations, and the rest of the activity could be eluted with 0.1 M KCl. These results demonstrate that host factor is selectively retained by binding to replicase-Sepharose.

Physicochemical Properties of Host Factor—The physicochemical properties of host factor are summarized in Table IV. Details are provided in Miniprint.

Properties of the Host Factor-stimulated Reaction—Stim-
ulation of replicase activity by host factor is dependent upon the initial concentration of the host factor: the most highly concentrated host factor preparations are the most active, and for Fraction V host factor at saturating levels, we have observed from 20- to 60-fold stimulation above background.

At low concentrations of host factor, the rate of synthesis is proportional to host factor concentration (data not shown). A typical time course for the host factor-dependent replicase reaction (saturating host factor) is presented in Fig. 5. The reaction is linear for almost 60 min. The oligo(U)-stimulated replicase reaction displays virtually identical kinetics at the optimum of about 30 mol of oligo(U)/mol of RNA (Fig. 5).

Replicase activity increases with increasing amounts of host factor as well as with increasing replicase (Fig. 6A) or poliovirus RNA (Fig. 6B). However, the concentration of host factor required for maximal rate of synthesis appears to be independent both of replicase and RNA concentrations. The amount of host factor required for half-maximal activity was very nearly the same over a 4-fold range of (a) replicase concentrations when the amount of RNA in the reaction was held constant and (b) RNA concentrations when the amount of replicase was held constant. The experiment shown in Fig. 6B suggests that host factor does not interact strongly with poliovirus RNA. Indeed, no more than 10% of any given sample of Fraction IV host factor was able to bind to either phosphocellulose or denatured calf thymus DNA-cellulose.

Table IV

<table>
<thead>
<tr>
<th>Property</th>
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<tr>
<td>Molecular weight</td>
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<tr>
<td>Sodium dodecyl sulfate-polyacrylamide gel electrophoresis</td>
<td>67,000</td>
</tr>
<tr>
<td>Gel filtration</td>
<td>65,000</td>
</tr>
<tr>
<td>Sedimentation velocity</td>
<td>6.7 x 10^{-3} cm^2 s^-1</td>
</tr>
<tr>
<td>Stokes radius</td>
<td>32 Å</td>
</tr>
<tr>
<td>Sedimentation coefficient</td>
<td>4 S</td>
</tr>
<tr>
<td>Frictional ratio, f/f₀</td>
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</tr>
</tbody>
</table>

*Calculated from sedimentation through glycerol gradients, according to the procedure of Martin and Ames (31).

*Value obtained in gel filtration study using empirical standard curve.

*Calculated according to the procedure of Siegel and Monty (36).

*Estimated by assuming $\bar{v} = 0.73$ ml/g and using the empirically determined value for the Stokes radius and the molecular weight estimated from sedimentation velocity.

Fig. 5. Time courses of the host factor- and oligo(U)-stimulated replicase reactions. A saturating amount (3 μl) of Fraction V host factor (●) or the optimal amount (6 pmol) of oligo(U) (△) was added to an assay mixture containing 0.6 μg of poly(U) polymerase purified by gradient elution from poly(U)-Sepharose 4B and 0.5 μg of poliovirus RNA in a total volume of 50 μl (50 mm HEPES-KOH, pH 8, 4 mm dithioerythritol, 10 μg of actinomycin D/ml, 3.5 mM Mg-aceate, 0.1 mM ZnSO₄, 3.5 mM [³H]CTP, and 0.2 mM each of unlabeled ATP, GTP, and UTP). Reactions (performed in duplicate) were allowed to proceed at 30 °C for the indicated times and were then quenched by the addition of sodium pyrophosphate. Incorporation of [³H]CMP (11,400 cpm/pmol) into acid-precipitable polymer was determined by liquid scintillation counting. The incorporation of radioactivity by poly(U) polymerase in the absence of host factor or oligo(U) is also indicated (■).

Fig. 6. Dependence of replicase activity on the concentration of host factor, replicase, and poliovirus RNA. Assays were performed in a total volume of 50 μl (50 mm HEPES-KOH, pH 8, 4 mm dithioerythritol, 10 μg of actinomycin D/ml, 3.5 mM Mg-acetate, 0.1 mM ZnSO₄, 3.5 mM [³H]CMP, and 0.2 mM each of unlabeled ATP, GTP, and UTP). Samples were incubated for 45 min at 30 °C and incorporation of [³H]CMP (11,800 cpm/pmol) into acid-precipitable radioactivity was measured. Background incorporation in the presence of poly(U) polymerase alone was less than 5% and was subtracted. A, Fraction IV host factor was added to assay mixtures containing 1 μg of poliovirus RNA and 0.45 μg (●), 0.9 μg (○), or 1.8 μg (■) of replicase purified by gradient elution from poly(U)-Sepharose 4B. B, Fraction IV host factor was added to assay mixtures containing 0.6 μg of replicase purified by gradient elution from poly(U)-Sepharose and 0.5 μg (○), 1 μg (△), or 2 μg (■) of poliovirus RNA.

Fig. 7. Template specificity of host factor-dependent replicase. Poly(U)-Sepharose-purified replicase (0.6 μg) was incubated with a saturating amount (3 μl) of Fraction V host factor and the indicated number of micrograms of each template RNA for 45 min at 30 °C in a total volume of 50 μl (50 mm HEPES-KOH, pH 8, 4 mm dithioerythritol, 10 μg of actinomycin D/ml, 3.5 mM Mg-acetate, 0.1 mM ZnSO₄, 3.5 mM [³H]CTP, and 0.2 mM each of unlabeled ATP, GTP, and UTP). Incorporation of [³H]CMP (10,000 cpm/pmol) into acid-precipitable form was determined by liquid scintillation counting. The RNA templates were purified from poliovirus (●), cowpea mosaic virus (△), alfalfa mosaic virus (○), and brome mosaic virus (△).
even at pH 6.8 and in the absence of salt, and irrespective of the presence or absence of Mg\(^{2+}\) (data not shown), suggesting that the host factor is unlikely to be a general nucleic acid-binding protein.

In the presence of saturating Fraction V host factor, there is a slight preference for poliovirus RNA over other viral RNAs (Fig. 7). In the experiment of Fig. 7, a saturating amount of host factor was used; however, virtually identical results were obtained using limiting amounts of host factor (not shown). Under the reaction conditions described here, host factor stimulates the synthesis of a faithful copy of template RNA (see accompanying report, Ref. 33). Our most highly purified preparations of host factor and poly(U) polymerase are capable of synthesizing full length (35 S) molecules of RNA (see accompanying report).

**DISCUSSION**

We have shown here that the activity of poliovirus replicase 
*in vitro* is dependent upon the presence of a soluble host cell protein. The cytoplasmic location of this protein, previously termed "host factor" (16), is consistent with the fact that all template-dependent poliovirus replicase preparations reported to date have also been cytoplasmic (2, 5, 16, 34). The presence of only about 20% of host factor activity in ribosomes, and then only in loose association, is consistent with the lack of evidence for host factor activity in highly purified preparations of any of the known protein synthesis initiation or elongation factors (16).

A scheme is presented for the isolation and several thousand-fold purification of host factor with good yields. Conditions have been obtained under which even the most highly purified host factor preparations are stable for many months. On sodium dodecyl sulfate-polyacrylamide gel electrophoresis, two protein bands are detectable by staining with Coomassie brilliant blue, but only one of the two proteins co-elutes with host factor activity during the final step of purification. The approximate molecular weight of the latter protein, determined under denaturing conditions, is 69,000, consistent with estimates of 67,000 from gel filtration and 65,000 from sedimentation velocity experiments. Taken together, the various hydrodynamic properties of the host factor (summarized in Table IV) suggest that the protein is globular. The host factor is a slightly acidic protein with an isoelectric point in the neighborhood of 6.3. On the basis of our estimated yield of the 69,000-dalton protein, there are probably 50,000-100,000 molecules/cell (0.003-0.006% of total cellular protein).

In the original identification of a host cell protein requirement for replicase activity 
*in vitro*, it was assumed, but not proven, that partially purified replicase preparations were contaminated with host factor; the "contaminant" was evidently removed by subsequent gradient elution from poly(U)-Sepharose (16). The salt concentration at which host factor activity elutes has now been identified, and replicase activity has been reconstituted by combining this host factor activity with poly(U)-polymerase from the same column. This result strongly suggests that replicase itself has not simply been altered during purification and that host factor activity was indeed present in the starting material. In the accompanying report (33), we demonstrate that our most highly purified preparations of host factor and poly(U) polymerase display a replicase activity, the product of which is a full-length (35 S) copy of the positive strand template RNA. Host factor activity is also required for copying of negative strand RNA.3

Although there is a preference for poliovirion RNA as template, it is not as striking as that reported earlier (16). In particular, the host factor isolated from ribosomes showed a stronger preference for poly(A)-containing templates. The reason for the discrepancy is unclear, but could be related to the different sources of the host protein. Alternatively, the most highly purified replicase used in these experiments may be deficient in a protein required for specific copying of the RNA template.

Experiments in which host factor is titrated against different amounts of replicase (RNA concentration constant) or different amounts of RNA (replicase concentration constant) seem to indicate a lack of any strong interaction with either poly(U) polymerase or RNA. This result is in contrast with findings for host factor I of phage Qb which by similar experiments was shown to interact with RNA (11). The latter host factor is not found as a subunit of highly purified Qb replicase, but as pointed out by Carmichael et al. (35), this observation does not preclude the existence of a loose association with the replicase, and support for this viewpoint is cited. In the case of the poliovirus replicase, there is to date no evidence for a subunit structure. The same argument concerning physical interactions between host factor and polymerase can be invoked here, and we present evidence for weak association: host factor is selectively retained on a column of replicase-Sepharose. The very small amounts of the replicase protein p63 present in highly purified preparations severely limit the practical utility of this type of affinity chromatography as a purification step for host factor. The experiment does, however, indicate a weak association between host factor and the replicase protein.

Van Dyke and Flanagan (5) have developed a purification scheme for poly(U) polymerase which involves ammonium sulfate fractionation of a 200,000 × g supernatant (S200) followed by phosphocellulose chromatography, gel filtration on Sephacryl S-200, and hydroxylapatite chromatography. Before chromatography on phosphocellulose, the polymerase was found to sediment at 6 S (5), but on further purification, a sedimentation coefficient of 4 S was obtained. The implication of this result is that in the 6 S form, p63 is complexed with one or more macromolecular factors which are separated away in later chromatographic steps. This factor (or factors) is clearly not required for elongation, because the 4 S form of replicase can synthesize heteropolymers of RNA in the presence of oligo(U). It is not known whether this 4 S enzyme would be active if host factor were substituted for oligo(U). However, if the 8 S form of the enzyme represented a replicase-host factor complex, our own conclusions drawn from affinity chromatography would be supported.

The development of a scheme for the purification of stable, high specific activity host factor is essential for more detailed studies on the function of the poliovirus replicase. We show, in the accompanying report, that highly purified protein fractions of host factor and poliovirus replicase can be combined 
*in vitro* in a reaction which faithfully mimics the synthesis in 
*in vivo* of full length complementary RNA.

This fully reconstituted system has made it possible for us to investigate the 5'-terminal structure of the replicase reaction product.4 It has been suggested that the small, virus-encoded protein (VPg) found in covalent linkage at the 5'-termini of both positive and negative strand RNA molecules may function as a primer for RNA synthesis (36-39). We have used chemically synthesized VPg peptides to raise antibodies which react with authentic VPg as well as with higher molecular weight, VPg-related polypeptides (18). Experiments with these antibodies to be reported elsewhere\(^5\) suggest that VPg or a VPg-related polypeptide is associated with the RNA

\(^4\) M. H. Baron and D. Baltimore, manuscript in preparation.
products of the in vitro replicase reaction. Anti-VPg antibodies can inhibit the host factor-stimulated replicase reaction but not the oligo(U)-stimulated reaction, implying that both a VPg-related polypeptide and host factor are involved in the initiation of the replicase reaction.

REFERENCES