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Expression of enzymatically active poliovirus RNA-dependent RNA polymerase in *Escherichia coli*

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ABSTRACT **The poliovirus genome is replicated by a virus-encoded RNA-dependent RNA polymerase (RNA polymerase). The RNA polymerase is first synthesized as a larger precursor polypeptide, which is subsequently processed by a viral proteinase, 3C^pro^, to give the mature polymerase molecule, 3D^pol^. To further characterize the poliovirus RNA polymerase, we have constructed plasmids that expressed this protein in *Escherichia coli*. The plasmids consisted of fusions between the *E. coli* DNA encoding the first 13 amino acids of the *trp* operon leader protein and viral genes encoding the 3C^pro^ and 3D^pol^ polypeptides. *E. coli* harboring such plasmids gave significant, inducible levels of enzymatically active RNA polymerase as determined by the poly(A)·oligo(U) poly(U) polymerase assay. The purified RNA polymerase activity from *E. coli* corresponded to a protein with the approximate molecular weight of the mature 3D^pol^ protein. The availability of a recombinant, enzymatically active poliovirus RNA polymerase provides a system in which we can precisely delineate the role this enzyme plays in the regulation of poliovirus replication.**

The single-stranded RNA genome of poliovirus is replicated by a virus-specific RNA-dependent RNA polymerase (RNA polymerase) (1). Previous studies have reported the purification of the poliovirus RNA polymerase from the membranes (2) and cytoplasm of poliovirus-infected cells (3). The purified RNA polymerase corresponds to a single molecule of ≈52 kDa (4, 5).

The poliovirus RNA polymerase is initially synthesized as a large polyprotein that constitutes the entire translation product of the poliovirus genome (6–8). The mature RNA polymerase, or 3D^pol^ [named as suggested by Rueckert and Wimmer (9)], is generated by cleavage of this polypeptide at a specific glutamine-glycine bond by a viral proteinase (3C^pro^) (7, 10). The availability of cDNA clones of the poliovirus genome has provided the opportunity to use molecular genetics to analyze this process in detail (11–13). Studies by Hanecak *et al.* (14) and Ivanoff *et al.* (15) have shown that the viral proteinase, when expressed in *E. coli*, will accurately process the poliovirus polyprotein at glutamine-glycine residues, with one of these cleavages corresponding to the correct junction between the 3C^pro^ and 3D^pol^ molecules.

To gain a better understanding of the activity of the RNA polymerase in poliovirus replication, we have sought to express this molecule in *Escherichia coli*. We report that we have generated constructs that enable the expression of an enzymatically active RNA polymerase in *E. coli*. To do this, we have taken advantage of the fact that the viral proteinase is enzymatically active when expressed in *E. coli* and have made constructs that encode a polyprotein composed of the viral proteinase 3C^pro^ and the polymerase 3D^pol^ molecules. We have partially purified the induced RNA polymerase activity from *E. coli* and show that this activity correlates with a protein of approximately 52 kDa, which is consistent with the notion that correct processing of the expressed polyprotein has occurred in *E. coli*.

MATERIALS AND METHODS

Bacteria and Plasmids. *E. coli* strain HB 101 (*recA13*$^-$, *hsdR*$^-$, *lacYl*, *supE44*) was used as a host for the experiments. The *trp* expression vector, pVVI, was obtained from C. Yanofsky, Stanford University (16). The cDNA clone of poliovirus, pPOV300 (containing the poliovirus sequence of nucleotides 4600–7400) was obtained from E. Ehrenfeld, University of Utah (13). A second polio cDNA clone, pMV7-2.9, was a gift from B. Semler, University of California, Irvine, and also contains the polio sequence of nucleotides 4600–7400.

DNA Manipulations. Restriction enzymes and T4 DNA ligase were used according to the manufacturer's instructions (Boehringer Mannheim) and as described in Maniatis *et al.* (17). DNA fragments were purified by using NA-45 paper as suggested by the manufacturer (Schleicher and Schuell).

Preparation of Lysates from *E. coli* and Partial Purification of the Induced Poliovirus Replicase. Overnight cultures of *E. coli* containing the poliovirus RNA polymerase plasmid constructs were grown in LB medium supplemented with 50 μg of tryptophan and 100 μg of ampicillin per ml. For induction, these cultures were diluted 1:100 in M9 medium with 1% Casamino acids and ampicillin (100 μg/ml). The cultures were grown for 3 hr ($OD_{600} \approx 0.1$). The *trp* operon inducer 3β-indoleacrylic acid was added at 10 μg/ml, and the cultures were allowed to grow for an additional 3–4 hr with vigorous shaking; the induced cultures were then harvested by centrifugation and stored at −70°C until use. The induced *E. coli* cell pellets were lysed by the procedure of Klempnauer *et al.* (18). For purification of the RNA polymerase from induced *E. coli*, the crude (20,000 × *g*) supernatant was first applied to a phosphocellulose column prepared as described by Dasgupta *et al.* (3). Further purification of the RNA polymerase was obtained by chromatography on an ACA 44 column (LKB, Uppsala, Sweden) (10 × 24 cm) standardized by chromatographing proteins of known molecular weight. In some instances, additional purification of the RNA polymerase was obtained by using hydroxylapatite chromatography as described by Van Dyke and Flanegan (5). The purified RNA polymerase was stored at −70°C and retained activity for at least six months. Protein was determined by using the Bio-Rad Assay Kit as instructed by the manufacturer (Bio-Rad).

Assay for RNA Polymerase. The poly(A)·oligo(U) poly(U) polymerase assay for RNA polymerase activity was performed as described by Flanegan and Baltimore (2) except that rifampin (20 μg/ml) was substituted for actinomycin D. The *in vitro* synthesized product was precipitated by 10%

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Abbreviations: RNA polymerase, RNA-dependent RNA polymerase; Blotto, bovine lacto-transfer-technique optimizer.
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trichloroacetic acid with 100 μg of carrier tRNA in 0.2 M sodium pyrophosphate, collected on 0.45-μm Gelman filters, dried, and solubilized in Bray's solution; radioactivity was determined in a scintillation counter.

Metabolic Labeling of *E. coli*. Bacteria were labeled at the end of induction with 50 μCi (1 Ci = 37 GBq) of [^{35}S]methionine for 20 min at 37°C with vigorous shaking. The bacteria were then pelleted, washed three times with phosphate-buffered saline, and lysed. The labeled proteins were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis (19). After electrophoresis, the gel was fixed, dried, and autoradiographed with Kodak X-AR film and a DuPont Cronex intensifier. In some instances the gels were also silver-stained by the method of Oakley *et al.* (20).

Labeling of Poliovirus-Infected HeLa Cells. Hela cells ($\approx$4 $\times 10^8$) were infected with poliovirus and labeled with 500 μCi of [^{35}S]methionine as described (19).

Electrophoretic Blotting of Polypeptides from Induced *E. coli*. Protein preparations from various stages of RNA polymerase purification were separated by $NaDodSO_4$/gel electrophoresis and electrophoretically transferred to nitrocellulose (21). After transfer, the blots were soaked in bovine lacto-transfer-technique optimizer [5% nonfat dry milk in phosphate-buffered saline with 0.1% sodium azide; Blotto (21)] to saturate the additional binding sites. The blot was then treated with rabbit anti-3D^{pol} antibody (kindly provided by O. Richards, University of Utah) appropriately diluted in Blotto. After washing with Blotto to remove unbound antibody, the blot was treated for 2 hr at room temperature with ^{125}I-labeled protein A prepared as described (22). The blot was then thoroughly washed with Blotto, dried, and autoradiographed at −70°C with Kodak X-AR film and a DuPont Cronex intensifier.

RESULTS

Construction of the Plasmid for the Expression of the Fusion Protein Between the *trp* Operon Leader Protein and 3D^{pol}. Since the gene order of poliovirus is such that the 3C^{pro} molecule directly precedes the RNA polymerase molecule, the autocatalytic action of the 3C^{pro} precursor should result in the correct amino terminus of the polymerase molecule (14, 15). Thus, we reasoned that expression of a fusion polyprotein consisting of the viral proteinase and RNA polymerase in *E. coli* should result in the appropriate processing and possibly folding of the polymerase molecule required for enzyme activity. The construction of the plasmid is presented in Fig. 1. The final plasmid, pPROT-POL, was identical to the poliovirus cDNA clones except that a *Bam*HI site had been created at position 5240, which had been a *Hin*dII site. To modify this plasmid for expression of the viral polymerase, pPROT-POL was digested with both *Eco*RI and *Bam*HI. The 2.6-kilobase (kb) *Eco*RI–*Bgl* II fragment of pVVI (16) was isolated and ligated with the digested pPROT-POL. The resulting plasmid, pPROT-POL-TRP, contained the *trp* operon with DNA encoding 13 amino acids of the leader protein fused to the poliovirus genomic sequences at the *Bam*HI site such that the reading frame was conserved (data not shown). The bacterial colonies harboring pPROT-POL-TRP were then screened for expression of the viral RNA polymerase.

Expression of Poliovirus RNA Polymerase in *E. coli*. Our initial attempts to detect RNA polymerase activity in crude lysates gave inconsistent results. We believe that this was due, in part, to the presence of numerous nucleases in the crude extracts. However, we were able to identify several potential positive colonies from which extracts gave significantly higher RNA polymerase activity than those from control extracts—i.e., those prepared from the vector with the *trp* regulatory regions minus the poliovirus sequences.

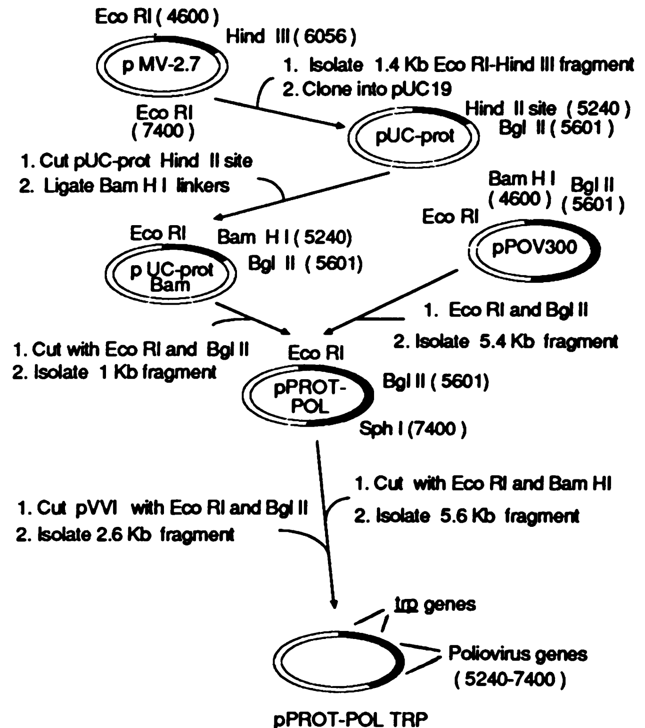

FIG. 1. Construction of *trp*-poliovirus fusions. An *Eco*RI-to-*Hin*dIII 1.4-kilobase (kb) fragment of pMV7-2.9 [nucleotides 4600–6056 of the poliovirus genome; areas in black represent poliovirus sequences; numbers in parentheses refer to the poliovirus nucleotide sequence (6)] was subcloned into pUC19 (23). This plasmid, named pUC-prot, was digested with *Hin*dII, followed by the addition of *Bam*HI linkers (New England Biolabs); the resulting plasmid was named pUC-prot-BAM. The 1-kb *Eco*RI–*Bgl* II fragment of pUC-prot-BAM was isolated and cloned into pPOV300 (13). The expression vector, pVVI (16), was digested with *Eco*RI and *Bgl* II, and the 2.6-kb fragment containing the *trp* operon operator-regulatory region was isolated. The plasmid, pPROT-POL, was digested with restriction enzymes *Eco*RI and *Bam*HI, and the 5.6-kb fragment was isolated and ligated with the *Eco*RI–*Bgl* II *trp* fragment to give the final plasmid, pPROT-POL-TRP.

Cultures of 500 ml were grown from colonies of these putative positive clones as well as the vector control and were starved for tryptophan in the presence of the *trp* inducer 3β-indoleacrylic acid (16). The crude extracts were then initially purified by phosphocellulose column chromatography. Fractions from the phosphocellulose column containing RNA polymerase activity were identified by the poly(A)·oligo(U) assay. Flanegan and Baltimore (2) have demonstrated that the RNA polymerase isolated from poliovirus-infected HeLa cells required Mg^{2+} for activity. RNA polymerase activity was readily detected in extracts purified from pPROT-POL-TRP; the RNA polymerase activity was dependent on Mg^{2+} (optimum at 2–3 mM), whereas extracts from bacteria harboring the vector alone gave background incorporation (data not shown). Additionally, Mn^{2+}, in the form of $MnCl_2$, did not substitute for Mg^{2+} in the *in vitro* reactions, a result that is consistent with that for the RNA polymerase isolated from poliovirus-infected HeLa cells (2).

Characterization of the RNA Polymerase Purified from Induced *E. coli*. The RNA polymerase activity detected in extracts purified from bacteria containing the pPROT-POL-TRP plasmids was first analyzed to determine the contribution, if any, of the *E. coli* DNA-dependent RNA polymerase to the poly(U) polymerase activity detected in our assays. To address this question, we assayed our extracts for sensitivity to rifampin, which is known to block the reinitiation of RNA chains by the *E. coli* DNA-dependent RNA polymerase

holoenzyme (24). Rifampin at 20 μg/ml had no effect on the poly(A)·oligo(U) poly(U) polymerase activity detected in extracts from pPROT-POL-TRP (Fig. 2*A*). In fact, we found that the addition of rifampin invariably stimulated the RNA polymerase activity in these preparations. The effect of rifampin on purified *E. coli* DNA-dependent RNA polymerase is shown in Fig. 2*B*. Surprisingly, the enzyme in the absence of rifampin had significant poly(A)·oligo(U) poly(U) polymerase activity. In the presence of rifampin at 20 μg/ml, however, this activity was completely inhibited.

The RNA polymerase activity detected in extracts from pPROT-POL-TRP was next characterized with respect to template and primer requirements for activity (Table 1). The RNA polymerase activity was completely dependent on the presence of both oligo(U) and poly(A) in the reaction mixture; omission of either one resulted in an almost complete loss of activity. Significant incorporation was also detected where oligo(dT) was substituted for oligo(U) in the reaction (data not shown), in contrast to earlier studies that demonstrated that the poliovirus RNA polymerase isolated from infected HeLa cells would not use oligo(dT) as a primer in the poly(U) polymerase assay (2). DNA polymerase I from *E. coli*, though, has been shown to have significant reverse transcriptase activity (25). However, no RNA polymerase activity was detected with a purified (commercial) preparation of the Klenow fragment of *E. coli* DNA polymerase I. Finally, as would be expected for the poliovirus RNA polymerase (3), the activity was sensitive to KCl.

Partial Purification of the Poliovirus RNA Polymerase Produced by *E. coli*. To further characterize the RNA polymerase activity in the bacteria harboring pPROT-POL-TRP, we purified the enzyme induced from *E. coli* according to standard procedures for the poliovirus RNA polymerase (3, 5). The crude lysate was first fractionated over a phosphocellulose column. Upon elution of the bound proteins with a linear salt gradient, RNA polymerase activity was readily detected from the extracts prepared from *E. coli* with pPROT-POL-TRP, whereas extracts from cells with the vector alone did not have any detectable activity (Fig. 3*A*). The material with RNA polymerase activity was eluted from the phosphocellulose column at a salt concentration of ≈0.15–0.2 M, which is consistent with that reported for the enzyme purified from poliovirus-infected cells (3). The peak fractions of RNA polymerase activity were pooled, concentrated, and applied to an ACA 44 molecular sieving column (Fig. 3*B*). The elution profile of the RNA polymerase activity was consistent with a protein having a molecular mass of ≈50,000–60,000 kDa, a value similar to that reported for the poliovirus RNA polymerase isolated from poliovirus-infected HeLa cells. Also, this molecular mass differs from that observed for the *E. coli* DNA polymerase I (109 kDa) and the *E. coli* holoenzyme DNA-dependent RNA polymerase (450 kDa) (26). Further purification of the recombinant RNA polymerase was then achieved by hydroxylapatite column chromatography (5). As judged by the poly(U) polymerase assay, we estimate that the recombinant RNA polymerase in the hydroxylapatite fraction has been purified >35-fold relative to the RNA polymerase activity in the crude extract. An analysis of the peak enzyme fractions from each step in the purification by silver-staining of $NaDodSO_4$/polyacrylamide gels revealed that a protein of 50–55 kDa copurified with the RNA polymerase activity (Fig. 4*A*). Several smaller molecular mass proteins were also evident in our purified enzyme preparations. At present, we do not know whether these proteins represent *E. coli* proteins, breakdown products of the poliovirus RNA polymerase, or alternative cleavage products of the processing of the polyprotein by the viral proteinase.

Further indication that the protein of ≈50–55 kDa purified from *E. coli* harboring pPROT-POL-TRP is the poliovirus

Table 1. Template and primer requirements for RNA polymerase activity purified from *E. coli* containing pPROT-POL-TRP

Template	Addition	UMP incorporated,* pmol/μg
pPROT-POL-TRP†	Poly(A), oligo(U)†	4.0
	Poly(A)	<0.1
	Oligo(U)	0.4
	None	0.2
pPROT-POL-TRP	50 mM KCl‡	2.0
	500 mM KCl‡	<0.1
E. coli DNA Pol I (Klenow fragment)§		
5 units		<0.1
10 units		<0.1

*The specific activity of the [α-^{32}P]UTP (Amersham) used in these experiments was 10,000 cpm/pmol.
†Phosphocellulose-purified RNA polymerase from induced *E. coli* harboring pPROT-POL-TRP.
‡A final concentration of 50 mM or 500 mM KCl was included in the complete poly(A)·oligo(U) poly(U) polymerase reaction mixture.
§Klenow enzyme units as defined by manufacturer (New England Biolabs). Standard reaction conditions for the poly(U) polymerase reaction, including poly(A) and oligo(U), were used at each concentration of Klenow enzyme tested. Pol I, polymerase I.

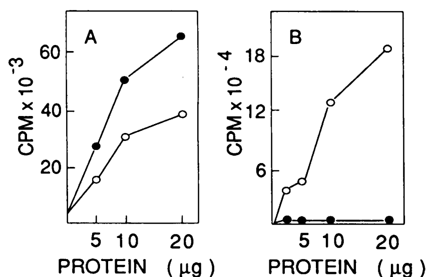

FIG. 2. Effect of rifampin on the recombinant poliovirus RNA polymerase activity. (*A*) Poly(U) polymerase activity of recombinant RNA polymerase in the presence of rifampin. Several concentrations of partially purified RNA polymerase from induced bacteria with pPROT-POL-TRP were tested for poly(U) polymerase activity in the absence (○) and presence (●) of rifampin at 20 μg/ml. (*B*) Partially purified *E. coli* DNA-dependent RNA polymerase (24) was tested for poly(U) polymerase activity in the absence (○) and presence (●) of rifampin at 20 μg/ml.

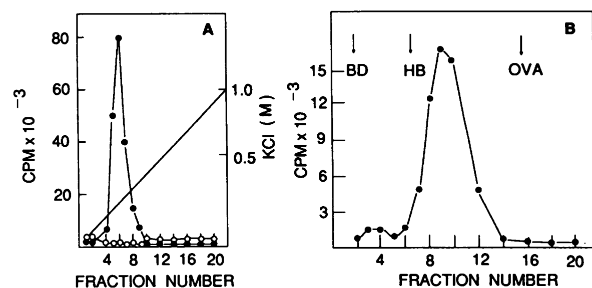

FIG. 3. Partial purification of the induced recombinant RNA polymerase. (*A*) Phosphocellulose chromatography. The supernatant from centrifugation at 20,000 × *g* from a 500-ml culture of induced bacteria harboring the plasmid pPROT-POL-TRP or the vector alone were assayed for poly(A)·oligo(U) poly(U) polymerase activity (●, pPROT-POL-TRP; ○, vector). (*B*) ACA 44 column chromatography. The fractions containing the peak poly(U) polymerase activity from the phosphocellulose column were applied to an ACA 44 column, and fractions were assayed for poly(U) polymerase activity (●). The column was standardized with blue dextran (BD; 3000 kDa), hemoglobin (HB; 64 kDa), and ovalbumin (OVA; 45 kDa).

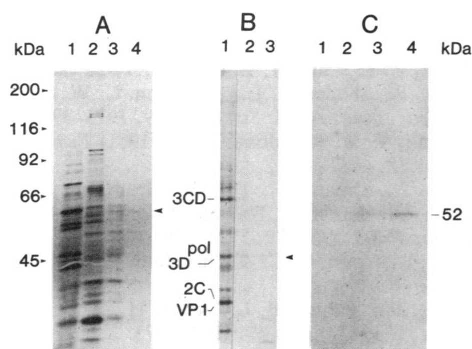

FIG. 4. Electrophoretic and immunoblot analysis of recombinant poliovirus RNA polymerase preparations. (*A*) Analysis of the purification of recombinant RNA polymerase by $NaDodSO_4$/gel electrophoresis. Approximately 20 μg of the samples at various stages of purification of the recombinant RNA polymerase were subjected to $NaDodSO_4$/gel electrophoresis followed by silver-staining. Lanes: 1, supernatant from centrifugation at 20,000 × *g*; 2, peak poly(U) polymerase pool of phosphocellulose column; 3, peak poly(U) polymerase pool from ACA 44 column; 4, peak poly(U) polymerase fraction from hydroxylapatite column. Molecular mass standards are as indicated, and the position of the putative $3D^{pol}$ molecule is marked. (*B*) Comparison of RNA polymerase expressed in *E. coli* with that from poliovirus-infected HeLa cells. Poliovirus-infected HeLa cells and induced bacteria with pPROT-POL-TRP were labeled with [^{35}S]methionine. The polypeptides were separated by $NaDodSO_4$/gel electrophoresis, followed by fluorography to visualize the labeled proteins. Lanes: 1, supernatant (20,000 × *g*) from poliovirus-infected HeLa cells; 2, phosphocellulose-purified RNA polymerase from poliovirus-infected HeLa cells; 3, supernatant (20,000 × *g*) from *E. coli* harboring pPROT-POL-TRP. The position of the 52-kDa $3D^{pol}$ molecule is marked. (*C*) Immunoblot analysis of recombinant RNA polymerase preparations. Protein preparations from induced *E. coli* containing the vector or pPROT-POL-TRP were separated on a resolving 15% $NaDodSO_4$/polyacrylamide gel and electrophoretically transferred to nitrocellulose. Immunoblots were then obtained with an anti-$3D^{pol}$ antiserum. Lanes: 1, high-salt fraction (40 μg); 2, phosphocellulose column fractions (40 μg) from *E. coli* containing the vector plasmid; 3, high-salt fraction (40 μg); 4, phosphocellulose fractions (40 μg) from *E. coli* containing pPROT-POL-TRP. Molecular mass markers were run on parallel lanes, and the position corresponding to 52 kDa is marked.

RNA polymerase was obtained by comparison of the migration of this protein on $NaDodSO_4$/polyacrylamide gels with the viral polymerase isolated from poliovirus-infected HeLa cells. The *E. coli* containing the pPROT-POL-TRP plasmid were labeled with [^{35}S]methionine at the end of induction, followed by partial purification of the RNA polymerase (20,000 × *g* supernatant). Poliovirus-infected HeLa cells were incubated with [^{35}S]methionine during infection, followed by purification of the RNA polymerase activity by phosphocellulose chromatography. Analysis by $NaDodSO_4$/polyacrylamide gel electrophoresis followed by autoradiography revealed that the purified protein from induced *E. coli* comigrated with the $3D^{pol}$ molecule purified from poliovirus-infected HeLa cells (Fig. 4*B*). Finally, an immunoblot analysis was utilized to confirm that the induced *E. coli* with pPROT-POL-TRP had expressed a mature $3D^{pol}$ protein (Fig. 4*C*). An antibody specific for poliovirus $3D^{pol}$ was used to detect the presence of $3D^{pol}$ in extracts prepared from induced *E. coli* containing the vector or pPROT-POL-TRP; the characterization of this antibody will be reported elsewhere (O. Richards, personal communication). The anti-$3D^{pol}$ antibody did not react with control extracts prepared from *E. coli* harboring the vector alone (Fig. 4*C*, lanes 1 and 2). A protein of 52 kDa was detected by the anti-$3D^{pol}$ antibody in both the crude extract (lane 3) and to a greater extent in the phosphocellulose column purified RNA polymerase (lane 4) prepared from *E. coli* containing pPROT-POL-TRP. The 52-kDa band comigrated with [^{35}S]methionine-labeled $3D^{pol}$ isolated from poliovirus-infected HeLa cells, which was electrophoresed in a parallel lane (data not shown).

DISCUSSION

The purpose of this study was to develop a system in which we could manipulate the poliovirus RNA polymerase gene by expressing its product in *E. coli*. The experiments described here show that the poliovirus RNA polymerase can be expressed in *E. coli* in an enzymatically active form that is stable and can be purified by standard methods as described for the isolation of the polymerase from poliovirus-infected HeLa cells (3, 5). Several lines of evidence were presented that show that the RNA polymerase activity measured from induced *E. coli* containing the pPROT-POL-TRP plasmid is due to the expression of the poliovirus RNA polymerase. First, the RNA polymerase activity is not detected in extracts prepared from *E. coli* with the vector alone or from extracts of induced *E. coli*. Second, the RNA polymerase activity detected in induced *E. coli* with pPROT-POL-TRP is insensitive to rifampin, which is known to inhibit the *E. coli* DNA-dependent RNA polymerase (24). Thus, we believe that the activity detected from extracts of *E. coli* with the PROT-POL-TRP specifically results from the expression of an enzymatically active poliovirus RNA polymerase.

To express the poliovirus RNA polymerase in *E. coli*, we had to generate constructs that included genomic sequences encoding the viral proteinase $3C^{pro}$ as well as the polymerase $3D^{pol}$. The plasmid constructs that contained only the poliovirus $3D^{pol}$ gene expressed large amounts of protein, but the purified preparations had little or no activity (data not shown). Several experiments support the conclusion that expression of a polyprotein consisting of the viral proteinase and polymerase polyprotein resulted in an active $3C^{pro}$ that, after processing, yielded a mature, enzymatically active $3D^{pol}$ molecule. First, the expressed RNA polymerase exhibits a purification profile from several different columns that is similar to the profile of the RNA polymerase found in poliovirus-infected HeLa cells. Preliminary results indicate that the recombinant RNA polymerase will bind to poly(U)-Sepharose affinity columns and that elution is at a salt concentration consistent with that observed for the enzyme isolated from poliovirus-infected HeLa cells. Second, the mass of a major protein in the partially purified preparations of the recombinant RNA polymerase is ≈50–55 kDa, which is consistent with the mass of the RNA polymerase purified from poliovirus-infected HeLa cells (4, 5). Third, the recombinant RNA polymerase exhibits several properties analogous to those observed for the enzyme isolated from poliovirus-infected HeLa cells: dependence on oligo(U) and poly(A) for poly(U) polymerase activity (6), a Mg^{2+} requirement for activity that cannot be substituted for by Mn^{2+} (2), and inhibition of activity by KCl (3). Fourth, when the *E. coli* are metabolically labeled with [^{35}S]methionine at the end of induction, a time at which the *trp* promoters on the multicopy plasmid will direct the synthesis of 20–30% of the total bacterial protein synthesis (16), a protein can be identified that comigrates on $NaDodSO_4$/polyacrylamide gels with the partially purified $3D^{pol}$ molecule isolated from poliovirus infected cells that had been incubated with [^{35}S]methionine. Finally, an immunoblot analysis of the RNA polymerase preparations from induced *E. coli* harboring pPROT-POL-TRP revealed that a protein of 52 kDa reacted with anti-$3D^{pol}$ antibodies. From these results, we conclude that the correct processing of the expressed polyprotein in *E. coli* had occurred, resulting in the generation of a mature, enzymatically active $3D^{pol}$ molecule. This conclusion is also supported by previous studies in which it was shown that the viral

proteinase will accurately process in *E. coli* the viral polyprotein at the correct glutamine-glycine bonds, one of these corresponding to the junction between the $3C^{pro}$ and $3D^{pol}$ genes (refs. 14 and 15; O. Richards, personal communication). Interestingly, Farmerie *et al.* (27) have reported that the expression of enzymatically active reverse transcriptase of the human immunodeficiency virus in *E. coli* was also dependent on the coexpression of the viral protease.

The expression of enzymatically active poliovirus RNA polymerase in *E. coli* provides a foundation for further experimentation on the mechanism of poliovirus replication. Preliminary studies indicate that recombinant RNA polymerase preparations will catalyze the *in vitro* copying of poliovirus virion RNA.

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