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Biochemistry

A unique deoxyguanosine triphosphatase is responsible for the OptA1 phenotype of *Escherichia coli*

(DNA replication/deoxyguanosine triphosphate/DNA binding protein/bacteriophage T7 gene *1.2* protein/tripolyphosphate)

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ABSTRACT **_Escherichia coli optA1_, a mutant unable to support the growth of T7 phage containing mutations in gene *1.2*, contains reduced amounts of dGTP. Extracts of _E. coli optA1_ catalyze the hydrolysis of dGTP at a rate 50-fold greater than do extracts of _E. coli optA+_. The dGTPase responsible for the increased hydrolysis has been purified to apparent homogeneity. Purification of the protein is facilitated by its high affinity for single-stranded DNA. By using this purification scheme an identical dGTPase has been purified from _E. coli optA+_. The purified proteins catalyze the hydrolysis of dGTP to yield deoxyguanosine and tripolyphosphate. The products of hydrolysis, chromatographic properties, denatured molecular mass of 56 kDa, N-terminal amino acid sequence, substrate specificity, and heat inactivation indicate that the proteins purified from _optA1_ and from _optA+_ cells are identical and identify the enzyme as the deoxyguanosine 5′-triphosphate triphosphohydrolase purified to homogeneity from wild-type _E. coli_ [Seto, D., Bhatnagar, S. K. & Bessman, M. J. (1988) _J. Biol. Chem._ 263, 1494–1499]. _OptA1_ cells contain ≈50-fold more active molecules of the 56-kDa dGTPase than do _E. coli optA+_ cells.**

Isolation of the *optA1* mutant of *Escherichia coli* was based on the assumption that the products of some genes of phage T7 were not essential for growth because one or more host proteins could substitute for them (1). *E. coli optA1* demonstrated no obvious phenotype other than its inability to support the growth of T7 gene *1.2* mutants; the *optA1* mutation is located at 3.6 min on the *E. coli* linkage map (1). Gene *1.2* of phage T7 is located at position 15.37 on the T7 chromosome (2, 3) and encodes a 10-kDa protein. Expression of gene *1.2* protein is subject to a form of posttranscriptional regulation (4); expression is dependent on the pattern of cleavage of mRNA at the RNase III recognition site immediately following gene *1.2*.

Characterization of *E. coli optA1* infected with T7 *1.2* mutant phage showed that T7 DNA synthesis terminated prematurely and the DNA was degraded (1). Additional insight came from studies with T4 phage mutants (5, 6). Certain mutations (antimutator phenotype) in T4 gene *43* (DNA polymerase) as well as mutations in the *dexA* gene (exonuclease) render it unable to grow on *E. coli optA1*.

What is the biochemical basis for these diverse effects of the *optA1* mutation on phage growth? We have shown that *E. coli optA1* cells have lower levels of dGTP (by a factor of 5) than do *optA+* cells (7); the levels of the other dNTPs are unchanged. Furthermore, after infection of *E. coli optA1* by T7 *1.2* mutant phage, the pools of dATP, dTTP, and dCTP increase from 10- to 40-fold, similar to that observed in an infection with wild-type T7. However, dGTP levels remain unchanged as compared to a 200-fold increase in the wild-type phage-infected cells. We have purified gene *1.2* protein to homogeneity from cells in which the cloned gene is overexpressed (8). Either gene *1.2* protein or dGTP restores DNA synthesis to extracts of *E. coli optA1* cells infected with T7 *1.2* mutants (7, 8). Thus, the defect in DNA synthesis in *optA1* cells infected with T7 1.2 mutant phage is caused by a deficiency of dGTP in the *optA1* strain, a defect that is overcome by the gene *1.2* protein.

Can a deficiency in dGTP levels also explain the inability of T4 *dexA* and T4 *CB120* mutants to grow on *E. coli optA1* (5, 6)? The *dexA* gene encodes an oligonucleotidase that, though not essential for growth, does participate in the degradation of host DNA (9). We have proposed that when the level of dGTP is low, as in an *optA1* host, degradation of the host DNA by the *dexA* nuclease is required to yield dGMP (7). T4 *CB120* carries a mutation in gene *43*, the gene for T4 DNA polymerase, that gives rise to an antimutator phenotype (10). The T4 *CB120* polymerase has a 10- to 100-fold increased rate of nucleotide turnover (11), a parameter that reflects the hydrolysis of incorporated nucleotides during DNA synthesis. Such an increase in nucleotide turnover leads to an increased demand for dNTPs, and in the infected *optA1* cells dGTP would become limiting.

The reduced levels of dGTP found in *E. coli optA1* prompted us to examine the fate of exogenously added dGTP in extracts. We show that extracts of *optA1* cells contain a 50-fold increase in an active deoxyguanosine 5′-triphosphatase (dGTPase). The properties of the purified protein lead us to conclude that it is identical to the dGTPase originally identified in preparations of *E. coli* DNA polymerase I by Kornberg *et al.* (12). Seto *et al.* (13) have recently characterized this enzyme purified from wild-type *E. coli* and find that the activity is less by a factor of 30 than in *optA1* strains.

MATERIALS AND METHODS

Bacterial Strains and Medium. *E. coli HR42* and *E. coli HR44 optA1* are isogenic except for *optA1* and have been described (1). Two times concentrated YT medium (2× YT medium) consists of 16 g of tryptone, 10 g of yeast extract, and 5 g of NaCl per liter.

Assay of dGTPase. The standard assay for dGTPase measures the formation of Norit-nonadsorbable radioactivity arising from [α-^{32}P]dGTP. The reaction mixture (100 μl) contained 50 mM Tris·HCl (pH 7.4), 12 mM $MgCl_2$, 25 mM NaCl, 50 μM [α-^{32}P]dGTP (30 cpm/pmol), and dGTPase. Dilutions of the enzyme were made in 40 mM Tris·HCl, pH 7.5/0.5 mg of bovine serum albumin per ml. Incubation was at 30°C for 30 min. The reaction was stopped by the addition of 0.9 ml of a solution consisting of 0.6 ml of 1 M HCl, 0.1 M $Na_4P_2O_7$, 0.02 M KH_2PO_4, 0.2 ml of a Norit A suspension (20% packed volume), and 0.1 ml of bovine serum albumin (5 mg/ml). The solutions were mixed and allowed to sit for 5 min at 0°C. After centrifugation, 0.5 ml of the supernatant was pipetted into Liquiscint (National Diagnostics, Somerville, NJ) and the radioactivity was determined. A unit of

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dGTPase is the amount causing the production of 1 nmol of Norit-nonadsorbable tri[^{32}P]polyphosphate in 30 min at 30°C.

Identification of Products of dGTPase Reaction. The Norit-nonadsorbable ^{32}P radioactivity formed in the dGTPase reaction was identified as tripolyphosphate by chromatography on a Mono Q column (Pharmacia FPLC) using a 0–0.4 M NaCl gradient in 25 mM Tris·HCl, pH 7.0/0.1 mM EDTA where it elutes together with an unlabeled tripolyphosphate marker. Tri[^{32}P]polyphosphate was also identified by electrophoresis on Whatman no. 3 MM paper at 20 V/cm in 0.05 M sodium citrate (pH 5.0). To identify the other product of the reaction, the ^{3}H-labeled product of a dGTPase reaction containing [^{3}H]dGTP was separated from unreacted dGTP on a Mono Q column. The radioactive product was then identified as deoxyguanosine on a C_{18} column (Waters Associates HPLC) where it eluted together with marker deoxyguanosine.

Other Materials. DEAE-Sephadex A-50 and dextran T500 were from Pharmacia. Carbowax PEG-8000 was obtained from Union Carbide (Greenville, SC). Single-stranded DNA cellulose was prepared (14) by using heat-denatured salmon sperm DNA (Sigma). All radiolabeled nucleotides were obtained from DuPont–New England Nuclear, and unlabeled nucleosides and nucleotides were from Pharmacia.

Other Methods. Polyacrylamide gel electrophoresis in the presence of 0.1% sodium dodecyl sulfate and protein determinations were as described (8). The amino acid composition and the N-terminal sequence of the dGTPase were determined by using a Beckman 6300 amino acid analyzer and an Applied Biosystems (Foster City, CA) model 470A protein sequenator, respectively, by M. Recny (Genetics Institute, Cambridge, MA).

RESULTS

dGTPase Activity in Extracts of *E. coli optA1*. Extracts of *E. coli optA1* catalyze the hydrolysis of [α-^{32}P]dGTP to yield Norit-nonadsorbable radioactivity at a rate 50-fold greater than do extracts of *E. coli optA*$^{+}$ (Fig. 1). The increased activity in extracts of *optA1* cells is specific for dGTP; the rates of hydrolysis of α-^{32}P-labeled dATP, dCTP, dTTP, and GTP are not only much lower than that seen with dGTP but are also similar to those observed in extracts of *optA*$^{+}$ cells (Table 1). The increased phosphatase activity in *E. coli*

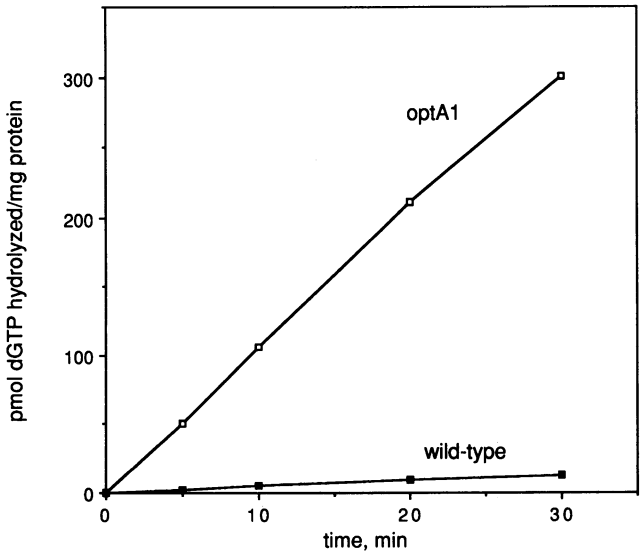

FIG. 1. dGTPase activity in extracts of *E. coli optA1* and *optA*$^{+}$ cells. Extracts were assayed in the standard dGTPase assay with 3 or 10 μg of protein per reaction mixture, respectively.

Table 1. Nucleoside triphosphatase activity in extracts of *E. coli optA1* and *optA*$^{+}$ cells

NTP	Activity, pmol of NTP hydrolyzed per 30 min/mg of protein	
	optA1	*optA*$^{+}$
dGTP	301	12.9
dATP	5.2	4.1
dCTP	4.2	6.4
dTTP	5.3	2.3
GTP	30	32

Extracts (3–10 μg of protein) of *E. coli optA1* and *E. coli optA*$^{+}$ were tested for their ability to hydrolyze nucleoside 5′ tri[α-^{32}P]phosphates in the dGTPase assay.

optA1 and its specificity suggest that it is responsible for the reduced levels of dGTP found in *optA1* cells (7).

Purification of dGTPase from *E. coli optA1*. After the cells were harvested, all procedures were carried out at 0–4°C. All buffers contained 0.1 mM EDTA except where indicated. Centrifugations were at 8000 × *g* for 20 min. The summary of the purification is presented in Table 2 and Fig. 2.

Growth of cells. Ten liters of *E. coli optA1* was grown in a New Brunswick (New Brunswick, NJ) fermenter at 37°C in 2× YT medium. At an A_{595} = 6, the cells were harvested by centrifugation, resuspended in 600 ml of 50 mM Tris·HCl, pH 7.5/10% sucrose/1 mM EDTA (buffer A), and again collected by centrifugation. The cells (60 g) were resuspended in buffer A and frozen in liquid N_2.

Preparation of cell extract. Frozen cells (30 g in 105 ml) were thawed at 0°C and 2.6 ml of 4 M NaCl and 2.1 ml of lysozyme (10 mg/ml) were added. After 60 min at 0°C, the cells were disrupted in 55-ml portions by sonication for 150 sec at a power setting of 6 in a model S75 Branson sonifier. The lysate was centrifuged and the supernatant was diluted to 500 ml by the addition of buffer A (fraction I).

Streptomycin sulfate fractionation. To 500 ml of fraction I was added 25 ml of 20% (wt/vol) streptomycin sulfate in buffer A over a 15-min period. After stirring for 1 hr, the precipitate was collected by centrifugation and suspended in 100 ml of 50 mM potassium phosphate buffer (pH 7.4) (buffer B). Insoluble material was removed by centrifugation and the supernatant was collected (fraction II).

Phase separation. Nucleic acids were removed by phase separation (16) as described by Seto *et al.* (13). Fraction II (115 ml) was made 5 M by the addition of solid NaCl, and then 14.5 ml of 60% (wt/vol) Carbowax PEG-8000 and 14.5 ml of 40% (wt/vol) dextran T500 were added to give final concentrations of 6% and 4%, respectively. The mixture was stirred for 90 min and then centrifuged to separate the phases. The lower phase containing the nucleic acids was discarded and the upper phase (90 ml) was dialyzed against 8 liters of buffer B. After clarification by centrifugation, $(NH_4)_2SO_4$ (0.2 g/ml) was added to the solution (210 ml) and it was transferred to a separatory funnel. After 7 hr, the lower phase was collected and dialyzed against 4 liters of buffer B containing 10% glycerol (fraction III, 248 ml).

DNA-cellulose chromatography. A column of single-stranded DNA cellulose (3.1 cm^2 × 7 cm) was equilibrated with buffer B containing 10% glycerol. Fraction III was applied at 100 ml/hr and washed with 50 ml of the same solution. The resin was washed with 60 ml of the buffer containing 0.57 M KCl and then washed with 60 ml containing 3.0 M KCl at 20 ml/hr. Fractions of 5 ml were collected; 5% of the activity was in the 0.57 M KCl fraction with 80% in three fractions of the 3.0 M KCl eluate. Fractions containing dGTPase activity were dialyzed against 4 liters of 20

Table 2. Purification of dGTPase from *E. coli optA1* and *optA*$^+$ cells

Fraction	Step	Protein, mg		Total units × 10^3		Specific activity, units/mg × 10^3		% recovery	
		optA1	*optA*$^+$	*optA1*	*optA*$^+$	*optA1*	*optA*$^+$	*optA1*	*optA*$^+$
I	Extract*	5900	7840	2210	47.6	0.37	0.006	100	100
II	Streptomycin sulfate	960	1120	1760	26.8	1.8	0.024	80	56
III	Phase separation	270	310	804	21.2	3.0	0.068	36	45
IV	DNA-cellulose	6.0	0.56	590	7.2	98	13	27	15
V	DEAE-Sephadex	5.6	0.10	570	3.0	102	30	26	6

*Extracts were prepared from 32 g of *E. coli HR44 optA1* and 40 g of *E. coli HR42* cells.

mM Tris·HCl, pH 7.6/20 mM NaCl/10% glycerol to yield 17 ml of fraction IV.

DEAE-Sephadex chromatography. A column of DEAE-Sephadex A-50 (0.8 cm^2 × 12 cm) was equilibrated with 20 mM Tris·HCl, pH 7.6/20 mM NaCl/10% glycerol. Fraction IV was applied at a flow rate of 9 ml/hr, and the resin was washed with 9 ml of the same buffer. Proteins were eluted with a 95-ml gradient from 20 to 320 mM NaCl in 20 mM Tris·HCl, pH 7.6/10% glycerol at a flow rate of 9 ml/hr. Fractions of 1.5 ml were collected; the peak of dGTPase activity eluted at 125 mM NaCl. Fractions containing dGTPase activity were pooled (20 ml) and dialyzed against 100 mM Tris·HCl, pH 8.0/100 mM NaCl/50% glycerol (fraction V).

Purification of dGTPase from *E. coli optA*$^+$. dGTPase has been purified from *E. coli HR42 optA*$^+$ by using the purification procedure developed for *E. coli optA1* cells (Table 2, Fig. 2). The only deviation from the procedure was during chromatography on DEAE-Sephadex A-50 where the column size was reduced to 0.8 cm^2 × 1 cm, the resin was washed with 3 ml of the equilibrating buffer, and the protein was eluted by using buffer containing 0.2 M NaCl in a volume of 1.5 ml.

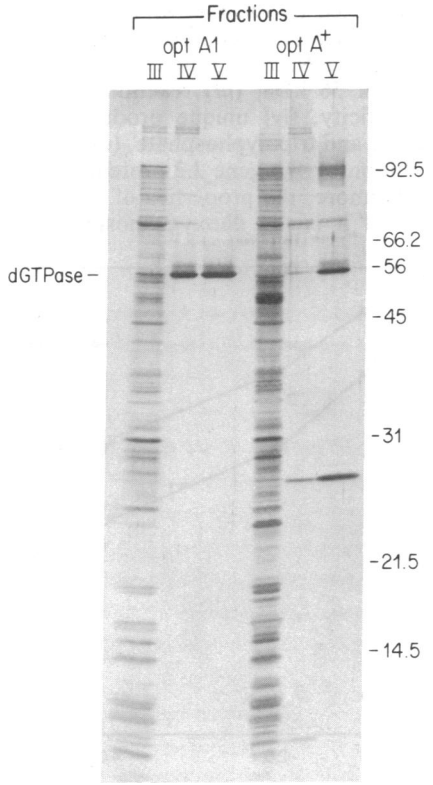

FIG. 2. Purification of dGTPase. Samples containing dGTPase were electrophoresed through a 10–25% gradient polyacrylamide gel containing 0.1% sodium dodecyl sulfate and the gel was stained with silver. Lanes III, IV, and V contain fractions III (10 μg), IV (1 μg), and V (0.9 and 1.8 μg, respectively) from the purification of dGTPase from *E. coli optA1* or *E. coli optA*$^+$ as indicated. Molecular mass standards (kDa) are indicated; the 56-kDa marker is T7 gene *4* protein (15).

Comparison of Purifications from *optA1* and *optA*$^+$ Cells. The dGTPase activity in extracts of *optA1* cells, although present in 50-fold excess over that found in *E. coli optA*$^+$, displays the chromatographic properties of the enzyme present in wild-type cells. Although a batch elution from DEAE-Sephadex was used for the purification of the enzyme from *E. coli optA*$^+$, in separate experiments, the enzyme was shown to elute at 125 mM NaCl, as does the enzyme from *optA1* cells. The purification yields proteins from *E. coli optA1* and *optA*$^+$ that are 100% and 30% pure, respectively, as judged by polyacrylamide gel electrophoresis (Fig. 2). When the purity of the enzyme from wild-type *E. coli* is taken into account, both proteins hydrolyze dGTP at the same rate (Table 2). The striking observation is that the amount of active dGTPase is increased 50-fold in *E. coli optA1*; a 275-fold purification yields a homogeneous protein from *optA1* cells compared to the 5000-fold purification required to obtain a protein that is only 30% pure from *optA*$^+$ cells.

The purification of dGTPase from *E. coli optA1* and *optA*$^+$ cells is facilitated by its high affinity for single-stranded DNA. The chromatography step on DNA cellulose yields an essentially homogeneous enzyme when the purification is carried out with *optA1* cells (Fig. 2). The lesser amount of the protein (by a factor of 50) in wild-type cells necessitates a second chromatography step, but the 200-fold purification on the DNA-cellulose column permits identification of the protein after this step (Fig. 2).

Physical Properties of the dGTPase. The molecular mass of the enzymes purified from *optA1* and *optA*$^+$ cells is 56 kDa as determined by gel electrophoresis in the presence of sodium dodecyl sulfate (Fig. 2). Seto *et al.* (13) have reported a molecular mass of 59 kDa for the denatured protein. The N-terminal amino acid sequence of the dGTPase from *E. coli optA1* shown below is identical to that of the 25 amino acids determined for the N terminus of the enzyme purified from wild-type cells (13). The amino acid composition of the dGTPase from *E. coli optA1* (data not shown) is similar to that reported for the enzyme from wild-type *E. coli* (13):

$$\overset{1}{\text{Ala}}\text{-Gln-Ile-Asp-}\overset{5}{\text{Phe}}\text{-Arg-Lys-Lys-Ile-}\overset{10}{\text{Asn}}\text{-Trp-His-Arg-}$$
$$\text{-Arg-}\overset{15}{\text{Tyr}}\text{-Xaa-Ser-Pro-Gln-}\overset{20}{\text{Gly}}\text{-Val-Lys-Thr-Xaa-}\overset{25}{\text{His}}\text{-Glu-}$$
$$\text{-Ile-Leu-Ile-}\overset{30}{\text{Ile}}\text{-Phe-Glu-Leu-Asp-}\overset{35}{\text{Xaa}}.$$

The enzymes purified from *E. coli optA1* and *optA*$^+$ lose activity during storage in the cold. At a protein concentration of 0.1 mg/ml, the enzyme has lost 20% and 60% activity at 0°C and −20°C, respectively, over 30 days. Since the enzyme is more stable at higher protein concentrations, the final chromatography step was altered so as to prevent dilution of the protein from wild-type *E. coli*, where it is present in relatively small amounts. Preparations that have lost 90% activity in the cold can be restored to full activity by

incubation at 25°C for 1 hr. The reversible loss of activity at low temperatures probably reflects the dissociation–reassociation of subunits, a phenomenon identified with other multisubunit enzymes (17). Seto *et al.* (13) have shown that the native enzyme consists of four homologous subunits.

Products and Stoichiometry of the dGTPase Reaction. The products of hydrolysis of dGTP catalyzed by the enzyme from *E. coli optA1* are deoxyguanosine and tripolyphosphate (Table 3). The identity of tripolyphosphate was established by electrophoretic analysis and by chromatography on a quaternary ammonium anion-exchange column. The other product was shown, by reverse-phase chromatography on a C_{18} column, to be deoxyguanosine. The loss of dGTP from the reaction was equal to the amount of deoxyguanosine and tripolyphosphate formed. The reaction is thus identical to that catalyzed by the enzyme from wild-type cells (12, 13).

Enzymatic Properties of the dGTPase. dGTPase from *E. coli optA1* cells displays the same substrate specificity as the dGTPase purified from wild-type cells (Table 4). Of the four dNTPs, only dGTP is hydrolyzed; neither dGDP nor dGMP is a substrate. GTP is hydrolyzed in the standard assay at ≈3% the rate of dGTP. The K_m values for dGTP and GTP are 2 μM and 110 μM, respectively, for the dGTPase purified from either wild-type *E. coli* or *E. coli optA1*. Kornberg *et al.* (12) had found the enzyme from wild-type cells to have a K_m of 2.5 μM for dGTP and 150 μM for GTP. Seto *et al.* (13) reported a K_m of 10 μM for dGTP.

Heat Inactivation. The dGTPase purified from *E. coli optA1* cells is relatively heat stable as is the enzyme from wild-type *E. coli* (13). Both enzymes maintain full activity (>90%) during incubation at 55°C for 60 min in the absence of substrate and $MgCl_2$; at 70°C, both enzymes lose >90% activity in 5 min (data not shown). The activities of the enzymes follow a similar heat inactivation curve (Fig. 3). After 60 min at 65°C, only ≈10% of the activity remained.

DISCUSSION

Our finding (7) that the *optA1* mutation in *E. coli* results in a specific decrease in the dGTP pool in uninfected cells and in cells infected with T7 gene *1.2* mutant phage explained the puzzling effect of the mutation on phage T7 and T4 growth (see Introduction). In considering the mechanism by which the *optA1* mutation lowered the dGTP pool we assumed that it must either affect the synthesis of deoxyguanosine nucleotides or a pathway that diverts them to reactions other than DNA synthesis (7). The greatly increased levels of dGTPase that we, as well as Seto *et al.* (13), find in extracts of *E. coli optA1* indicate that the latter explanation is the correct one.

Table 3. Products and stoichiometry of the dGTPase reaction

Time, min	dGTP, nmol	Deoxyguanosine, nmol	Tripolyphosphate, nmol
0	40.0	<0.7	<0.1
30	22.7	15.3	16.3
Δ	17.3	15.3	16.3

The reaction mixture (0.4 ml) contained 50 mM Tris·HCl (pH 7.4), 12 mM $MgCl_2$, 25 mM NaCl, 100 μM [^{3}H, α-^{32}P]dGTP (330 ^{3}H cpm/pmol; 6 ^{32}P cpm/pmol), and 20 units of dGTPase purified from *E. coli optA1* (fraction V). After incubation at 30°C for 30 min, the mixture was chilled to 0°C and made 200 mM with EDTA. An aliquot (50 μl) was treated as in the standard assay to determine Norit-nonadsorbable tri[^{32}P]polyphosphate. Another aliquot (10 μl) along with marker deoxyguanosine and dGTP were spotted on polyethyleneimine cellulose thin-layer plates (10 × 20 cm, Brinkmann) and developed in 1.0 M HCOOH/0.8 M LiCl. The deoxyguanosine- and dGTP-containing spots were visualized by UV light and cut out, and the amount of ^{3}H radioactivity in each was determined. The amount of dGTP, deoxyguanosine, and tripolyphosphate present at time zero was determined in an identical manner prior to incubation at 30°C.

Table 4. Substrate specificity of purified dGTPase

Nucleotide	Relative activity, % *optA1*	Relative activity, % *optA*$^+$
dGTP	100	100
dATP	<1	<2
dCTP	<1	<2
dTTP	<1	<2
dGDP	<1	<2
dGMP	<1	<2
GTP	3	2

Each of the indicated α-^{32}P-labeled nucleotides (50 μM) was tested in the standard dGTPase assay with fraction V of dGTPase purified from *E. coli optA1* or *optA*$^+$.

To characterize the dGTPase increased in *optA1* cells we have purified it to homogeneity. The purification requires two chromatography steps, one of which takes advantage of the high affinity of the enzyme for single-stranded DNA. By using this procedure we have purified the dGTPase, albeit at reduced levels, from wild-type *E. coli*. The purification obtained on DNA-cellulose alone is sufficient for identification of the dGTPase in wild-type cells by silver staining of polyacrylamide gels. The simple purification scheme combined with the high yield makes possible the examination of the enzyme in other strains and under different growth conditions.

The enzymes we have purified from *optA1* and *optA*$^+$ cells are indistinguishable. The identity of the two enzymes has been established by the following properties that they share: (*i*) chromatographic properties including high affinity for single-stranded DNA, (*ii*) a molecular mass of the denatured enzyme of 56 kDa, (*iii*) reaction requirements and substrate specificity, (*iv*) unique products of hydrolysis, deoxyguanosine and tripolyphosphate, (*v*) heat inactivation, and (*vi*) inhibition by the gene *1.2* protein of phage T7 (see below). Furthermore, the properties of the dGTPase leave little doubt that it is the deoxyguanosine 5′-triphosphate

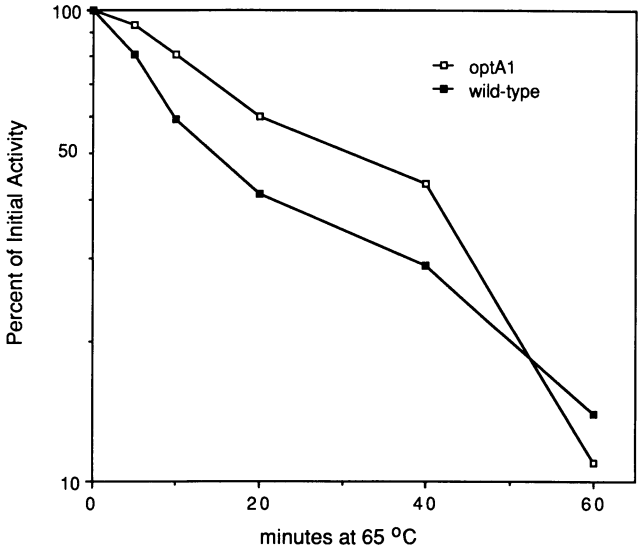

FIG. 3. Heat inactivation of dGTPase. Fraction V of dGTPase from either *E. coli optA1* or *optA*$^+$ was diluted into 50 mM potassium phosphate buffer, pH 7.4/0.1 mM EDTA to 200 units/ml and incubated at 65°C. At the times indicated, aliquots were withdrawn, diluted, and assayed for dGTPase activity in the standard assay. The percentage decrease in activity is relative to the zero time aliquots set at 100.

triphosphohydrolase previously described in wild-type *E. coli* (12, 13). In fact, the N-terminal amino acid sequence of the enzyme purified from *E. coli optA1* is identical to that reported for the enzyme purified from wild-type *E. coli* (13).

What is the basis of the 50-fold increase in dGTPase activity in extracts of *E. coli optA1*? It is clear (Table 2) that the increase in activity is a consequence of a 50-fold increase in the amount of active dGTPase protein in the *optA1* strain. Based on the purification and yield we estimate that *optA1* cells contain 10,000 molecules of the 56-kDa dGTPase per cell as compared to 200 active molecules per wild-type cell. Two explanations for this increase in the number of active molecules in the *optA1* strain are plausible. The most obvious is that the mutation affects the expression of the structural gene for dGTPase, the most likely candidate for the site of the mutation thus being a regulatory element involved in the transcription of the gene. A quite different model proposes that the *optA1* mutation resides within a structural gene that encodes a protein that inhibits the dGTPase. In this model, wild-type and *optA1* strains contain the same number of molecules of dGTPase, but 98% of the dGTPase molecules in wild-type cells go undetected in the dGTPase assay due to the presence of the hypothetical OptA protein inhibitor. T7 encodes just such an inhibitor of the dGTPase (see below). Other alternatives such as the *optA1* mutation residing in the dGTPase gene and affecting autoregulation by the dGTPase itself are less plausible.

The *optA1* mutant was isolated for its inability to support the growth of T7 phage defective in gene *1.2*. Our recent results show that the purified gene *1.2* protein is an inhibitor of the dGTPase purified from either *optA1* or *optA*$^+$ cells (H. E. Huber, B.B.B., and C.C.R., unpublished results), thus providing a biochemical basis for the requirement of the gene *1.2* protein during T7 infection of *optA1* strains. Although the gene *1.2* protein is not required for growth of the phage in wild-type *E. coli* we have not determined the amount of DNA or phage produced. Interestingly, whereas the dGTP pool is only reduced by a factor of 5 in *E. coli optA1* cells, it is reduced by a factor of 200 after infection with T7 gene *1.2* mutant phage (7). Thus, the 50-fold increased levels of dGTPase are far more manifest after phage infection. We suspect that a major portion of the dGTPase in uninfected *E. coli* does not have access to the dGTP pool. The dGTPase, although fully active, might be compartmentalized in a complex of other proteins bound to DNA. The breakdown of chromosomal DNA after T7 infection would result in the release of the dGTPase and hence the necessity for its inhibition by the phage. However, one should not conclude that its total elimination is desirable. The posttranscriptional control of expression of gene *1.2* (4) may ensure the proper level of dGTPase in the phage-infected cell.

The role of the dGTPase in *E. coli* is intriguing but unknown. Its properties, distinct from those of other phosphatases, make a compelling case for a specific role. (*i*) It is specific for dGTP, (*ii*) its activity, as evidenced by the *optA1* mutation, can vary over 50-fold, (*iii*) its products of hydrolysis are unique, (*iv*) it binds to DNA as strongly as do other proteins known to have specific roles in DNA metabolism, and, finally, (*v*) T7 encodes a protein, the gene *1.2* protein, that may regulate the level of dGTPase activity. At present there is no evidence to distinguish between a role of the enzyme modulating dGTP levels and one in which either tripolyphosphate or deoxyguanosine regulates another pathway. Alternatively, the preferred substrate for the enzyme may remain to be identified. Finally, the dGTPase activity observed could be the consequence of the uncoupling of a more complex reaction, perhaps resulting from the disruption of a multiprotein–DNA complex during purification.

Our own speculative view is that the dGTPase plays a role in *E. coli* and perhaps T7 DNA replication. In this capacity, its high affinity for single-stranded DNA suggests that it be bound at the replication origin during initiation or on the lagging strand at the replication fork. Our observations (H. E. Huber, B.B.B., and C.C.R., unpublished results) show that the dGTPase, when bound to DNA, is not only protected from inhibition by the T7 gene *1.2* protein but is actually more active. This result provides a mechanism by which excess dGTPase activity can be inhibited while maintaining an active enzyme bound to a DNA replicative intermediate, an intriguing mechanism if there were an OptA protein inhibitor of the dGTPase in *E. coli*. However, the relationship, if there is any, of the observed hydrolysis of dGTP to any of the known steps in DNA replication is a mystery.

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