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Characterization of the DNA-Protein Complex at the Termini of the Bacteriophage PRD1 Genome

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DNA of bacteriophage PRD1 has protein P8 at its termini. Extracts of infected cells are able to derivatize P8 in vitro with labeled dGTP. Two early proteins, P1 and P8, products of genes I and VIII, respectively, are the only phage proteins necessary for the formation of the protein P8-dGMP complex. This was shown by complementation of extracts from cells infected with mutants and by use of extracts from cells carrying cloned genes I and VIII. With *Escherichia coli* mutants that are temperature sensitive for DNA synthesis, it was possible to show that the formation of the protein P8-dGMP complex was dependent upon the host replication apparatus. The analysis of the purified protein P8-dGMP complex by hydrolysis and enzymatic digestion showed that there is a covalent phosphodiester bond between tyrosine and 5'-dGMP.

Bacteriophage PRD1 is a broad-host-range lipid-containing bacteriophage which can infect gram-negative hosts such as *Escherichia coli* and *Salmonella typhimurium* harboring an appropriate plasmid. For a more detailed description of this phage system, see Bamford et al. (4), Bamford and Mindich (3), McGraw et al. (15), and Mindich et al. (16, 17).

We have shown that there is a terminal protein strongly bound to the DNA of bacteriophage PRD1 (2). This protein is an early phage-specific protein designated P8. It is a product of gene VIII and is necessary for phage DNA replication. Infected-cell extracts catalyze the formation of a complex between protein P8 and [α-^{32}P]dGTP (2).

The relationship of the terminal protein to DNA synthesis appears to be analogous to that found in the cases of adenovirus (8, 12, 13, 27) and bacteriophage ϕ29 (10, 22).

In this study we have refined the conditions for the protein P8-dGMP reaction and have shown that extracts from cells containing cloned PRD1 genes I and VIII form the protein P8-dGMP complex much more effectively than do infected-cell extracts, that the terminal 5'-deoxyguanosine is covalently linked to tyrosine in protein P8 via a phosphodiester bond, and that the host cell replication apparatus is needed for the formation of this complex.

MATERIALS AND METHODS

Phages and bacteria. The bacterial strains used in this study are listed in Table 1. Plasmid pLM2 (18), which is necessary for phage adsorption, was transferred to *E. coli* JW1073 and RS116 by cross-streaking overnight broth cultures with cultures of *S. typhimurium* LT2(pLM2) and selecting for kanamycin and tetracycline resistance. For *E. coli* JW302 the donor was *Pseudomonas phaseolicola* HB10Y(pLM2), which is not capable of growing above ca. 34°C, and selection was for kanamycin resistance at 37°C. Plasmid clone 3, which has phage genes I and VIII (19) and is now designated pLM3, was introduced into *E. coli* JW101, JW107, JW110, JW111, JW112, JW176, JW177, JW180, JW267, and JW284 by transformation as previously described (19). Plasmid clone 141 is now designated pLM141. The transformants were expressed in L broth overnight at 26°C and selected for resistance to tetracycline and streptomycin over 2 nights at 29°C. The transformants obtained were purified and tested.

The phages used in this study are listed in Table 2. Nonsense mutants of PRD1 are designated with a mutant isolation number and a roman numeral that denotes the gene affected by the mutation. This number refers also to the name of the protein product of the corresponding gene; e.g., *sus2* contains (I) a mutation of gene I and is missing protein P1 in cells that do not suppress amber mutations. Phages PRD1, PR3, PR4, PR5, L17, PR722, and 12-1 were grown in LT2(pLM2), AP50 was propagated in nonpathogenic *Bacillus anthracis* CN18-74, and ϕ29 was propagated in *Bacillus subtilis* IS230. The bacteria in L broth at 37°C were infected at a multiplicity of infection of 10 at a cell density of ca. 5×10^8/ml. The phage concentration and purification were done as described by Bamford and Mindich (3), except that the final phage pellets were suspended into 50 mM Tris-hydrochloride (pH 7.5)–2.5 mM $MgCl_2$. The nonsense mutants of PRD1 were grown on appropriate suppressor hosts as indicated in Table 2. Purified single-stranded bacteriophage fd DNA was obtained from Loren A. Day, and bacteriophage ϕX174 DNA was obtained from Opinder S. Bhanot.

Preparation of cell extracts. Infected-cell extracts were made as described previously (2). Cells containing cloned PRD1 genes were grown in L broth containing 20 μg of tetracycline per ml to a density of ca. 5×10^8/ml at 37°C or, in the case of temperature-sensitive (*ts*) mutants, at 29°C and then processed as infected cells were.

Standard conditions for forming the protein P8-dGMP complex. Reaction mixtures (10 μl) contained 25 mM Tris-hydrochloride (pH 7.5), 20 mM $MgCl_2$, 1 mM ATP, 1 mM dithiothreitol, 1.5×10^{11} purified phage particles disrupted by heating them to 100°C for 20 s, 1 μM cold dGTP, 0.15 μCi of [α-^{32}P]dGTP (400 Ci/mmol; Amersham Corp.), and 4 μl of cell extract containing 20 mg of protein per ml. The standard conditions were 30°C for 75 s. The reactions were stopped, and the samples were prepared for protein gels as previously described (2). For reaction optimization, $MgCl_2$, ATP, phage, dGTP, and extract concentrations, as well as temperature and time, were varied as described below. When extracts from *ts* cells were used, the low-temperature reaction was run for 1 min at 30°C. The high-temperature

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TABLE 1. Bacterial strains used in this study

Strain	Relevant genotype or usage	Source or reference
S. typhimurium LT2(pLM2)	Wild type	16
S. typhimurium PSA(pLM2)	*sup*	16
S. typhimurium DB7156(pLM2)	*sup*	16
S. typhimurium MS1550(pLM2)	UV sensitive, nonsuppressor	16
P. phaseolicola HB10Y(pLM2)	Wild type	18
B. subtilis IS230	ϕ29 host	I.Smith
B. anthracis CN18-74	AP50 host	20
E. coli HB94(pLM2, pLM3)	Cloned PRD1 genes I and VIII	19
E. coli HB94(pLM2, pLM141)	Cloned PRD1 gene I	19
E. coli JW101(pLM3)	*dnaB*	J. A. Wechsler
E. coli JW107(pLM3)	*dnaB*	J. A. Wechsler
E. coli JW110(pLM3)	*dnaB*	J. A. Wechsler
E. coli JW111(pLM3)	*dnaE*	J. A. Wechsler
E. coli JW112(pLM3)	*dnaA*	J. A. Wechsler
E. coli JW176(pLM3)	*dnaC*	J. A. Wechsler
E. coli JW177(pLM3)	*dnaG*	J. A. Wechsler
E. coli JW180(pLM3)	*dnaC*	J. A. Wechsler
E. coli JW267(pLM3)	*dnaZ*	J. A. Wechsler
E. coli JW284(pLM3)	*dnaP*	J. A. Wechsler
E. coli JW302(pLM2)	*rep*	J. A. Wechsler
E. coli JW1073(pLM2)	*dnaA508*::Tn*10*, F	J. A. Wechsler
E. coli RS116(pLM2)	*dnaB252*::Tn*10*, P1 *ban*	23

treatment was done by incubating the extracts for 20 min at 37°C before running the reaction at 30°C for 1 min.

Purification and analysis of the protein P8-dGMP complex. The standard reaction was run with 35 times the normal volume (350 μl). Fifty microliters of 10× nuclease buffer (0.5 M sodium borate [pH 8.75], 5 mM $CaCl_2$) and 150 U of nuclease (*Staphylococcus aureus* nuclease; Boehringer Mannheim Biochemicals) were added to this mixture, and the mixture was incubated for 2 h at 37°C. The nuclease-digested mixture was run through a Sephadex G-25 column equilibrated with 0.1% sodium dodecyl sulfate (SDS)–50 mM sodium acetate, pH 7.5. The column fractions were counted and analyzed in protein gels. The protein P8-dGMP complex peak containing SDS was acetone precipitated and used in digestion studies. The acid hydrolysis was performed in 5.6 M HCl at 110°C for 90, 120, and 135 min. After hydrolysis, the HCl was evaporated overnight under reduced pressure, and the samples were suspended in water. Ten microliters each of phosphoserine, phosphothreonine, and phosphotyrosine (1 mg/ml) was added, and the samples were dried and analyzed by two-dimensional thin-layer electrophoresis as described by Collett et al. (6). The electrophoresis plates were stained with 0.5% ninhydrin in acetone and developed for 5 min at 110°C. Autoradiograms were prepared from the stained plates.

Enzymatic digestions of the acetone-precipitated purified protein P8-dGMP complex were performed as follows. To ca. 10,000 Cerenkov cpm of dried complex, enzymes and buffers were added to obtain a 20-μl final volume. The conditions for different enzymes were as follows: 2 μg of pronase in 30 mM Tris-hydrochloride (pH 8)–10 mM $MgCl_2$; 0.03 U of phosphodiesterase I (from snake venom; Sigma type VIII) in 30 mM Tris-hydrochloride (pH 8)–10 mM $MgCl_2$; 0.035 U of phosphodiesterase II (from bovine spleen; Sigma type I) in 50 mM sodium acetate, pH 5.2; and 2.5 U of alkaline phosphatase (from bovine intestine; Sigma type VII-L) in 30 mM Tris-hydrochloride (pH 8)–1 mM $MgCl_2$–0.01 mM $ZnCl_2$. The mixtures were incubated for 100 min at 37°C. A 3× protein gel sample buffer was added, and the samples were boiled for 2 min and analyzed in protein gels. The protein gels were 15.5% polyacrylamide, and the system was as previously described (16). The gels were dried, and autoradiograms were prepared. Visible bands were scanned, and the peak areas were calculated.

TABLE 2. Phage strains used in this study

Phage	Relevant genotype or usage	Host	Reference or source
PRD1	Wild type	LT2(pLM2)	16
PRD1	*sus2* (I)	PSA(pLM2)	16
PRD1	*sus38* (I)	PSA(pLM2)	16
PRD1	*sus233* (VIII)	DB7156(pLM2)	16
PRD1	*sus247* (VIII)	DB7156(pLM2)	16
PR3	Wild type	LT2(pLM2)	4
PR4	Wild type	LT2(pLM2)	4
PR5	Wild type	LT2(pLM2)	4
L17	Wild type	LT2(pLM2)	4
PR722	Wild type	LT2(pLM2)	5
N	Wild type	LT2(pLM2)	J. N. Coetzee
I2-1	Wild type	LT2(pLM2)	J. N. Coetzee
AP50	Clear plaque	CN18-74	20
ϕ29	Wild type	IS230	1

RESULTS

Forming the protein P8-dGMP complex with phage gene I and VIII products. By use of infected-cell extracts, disrupted phage particles, and [α-^{32}P]dGTP, it was possible to incorporate ^{32}P into protein P8 (2). This reaction was dependent upon added disrupted phage. Uninfected extracts did not have any activity. dGTP was the only nucleotide which was incorporated. The low activity of this reaction limited further characterization of the products. When the extract was made from cells containing cloned genes for PRD1 replication proteins P1 and P8 (pLM3 [15]), the efficiency of the dGTP derivatization of protein P8 increased at least 100 times. Figure 1 shows that protein P8 was labeled by radioactive dGTP (Fig. 1b and g) and that an extract of a plasmid-free

uninfected culture did not incorporate any nucleotide, although all nucleotides were labeled in the assay (Fig. 1f).

Phage mutants *sus2* and *sus38* in class I and mutants *sus233* and *sus247* in class VIII complement each other in a plaque assay. Extracts from LT2(pLM2) cells infected with these mutants were tested for their ability to form the protein P8-dGMP complex. The comparison was made with the activity achieved in a reaction with an extract of wild-type-infected cells (Fig. 2a). None of the mutants were able to form this complex alone (Fig. 2b, c, d, and e); however, extracts made from mutants *sus2* (I) and *sus233* (VIII) clearly complemented each other (Fig. 2f). Very weak complementation was seen with mutants *sus2* (I) and *sus247* (VIII) (Fig. 2g). No other complementation was observed.

Optimization of the protein P8-dGMP reaction. An investigation of reaction conditions revealed that the maximum amount of product was obtained after a 75-s reaction time at 30°C. The previously used 30-min reaction time resulted in a reduced amount of product (Fig. 3A). The reaction temperature did not seem to be very critical, but a slight peak appeared around 30°C (Fig. 3B). Because of the short reaction time, the reaction components were prewarmed shortly before mixing. The magnesium concentration for the maximum yield started at ca. 10 mM, but even four times that concentration did not have an inhibitory effect (Fig. 3E). The reaction proceeded without the addition of ATP, but a threefold stimulation was achieved at a concentration of ca. 1 mM (Fig. 3D). The addition of unlabeled dGTP up to a concentration of ca. 1 μM increased the yield (Fig. 3C).

Table 3 shows further reaction characteristics. The reac-

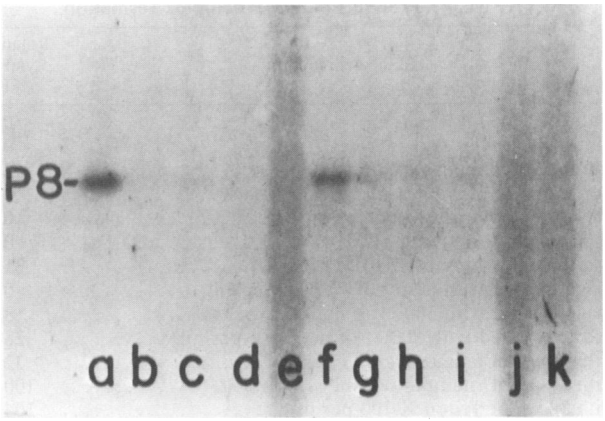

FIG. 2. Complementation assay for the P8-dGMP reaction with extracts from cells infected with nonsense mutants from classes I and VIII. Standard reaction with extract from cells infected with wild-type PRD1 (a). Reactions with extracts from cells infected with PRD1 mutants: (b) *sus2* (I); (c) *sus38* (I); (d) *sus233* (VIII); (e) *sus247* (VIII). Reactions with two extracts from cells infected with mutants: (f) *sus2* (I) and *sus233* (VIII); (g) *sus2* (I) and *sus247* (VIII); (h) *sus38* (I) and *sus233* (VIII); (i) *sus38* (I) and *sus247* (VIII); (j) *sus2* (I) and *sus38* (I); (k) *sus233* (VIII) and *sus247* (VIII).

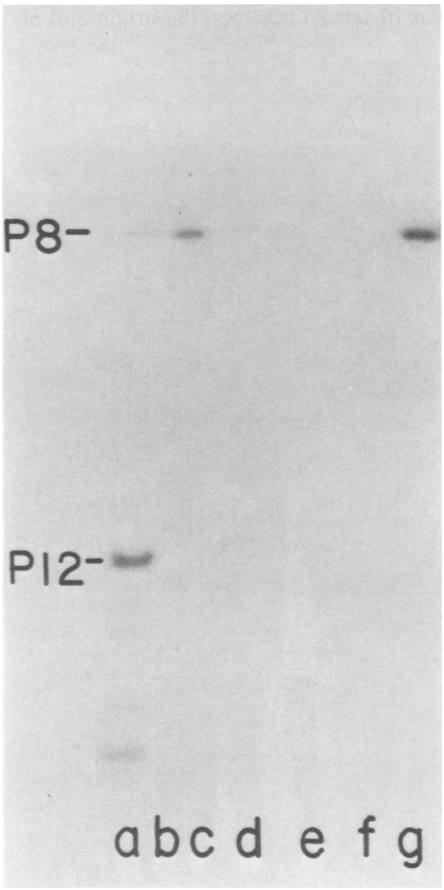

FIG. 1. Polyacrylamide gel electrophoresis of reaction products formed with extracts from cells containing cloned PRD1 genes I and VIII (pLM3). Samples were digested and analyzed on a 15.5% polyacrylamide gel. (a) [^{35}S]methionine-labeled early PRD1 proteins in UV-sensitive MS1550(pLM2) cells; (b and g) standard reaction with [α-^{32}P]dGTP; (c) [α-^{32}P]dCTP; (d) [α-^{32}P]dATP; (e) [α-^{32}P]dTTP; (f) [α-^{32}P]dGTP, [α-^{32}P]dATP, [α-^{32}P]dCTP, and [α-^{32}P]dTTP in a reaction mixture with extracts from cells without plasmid pLM3.

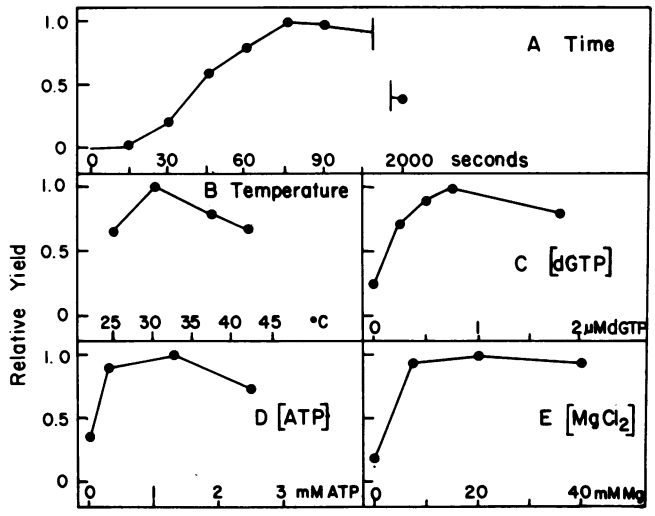

FIG. 3. Optimization of the protein P8-dGMP reaction. The reaction was run as described in the text. "Relative yield" on the ordinate refers to the relative intensity of the autoradiogram band for ^{32}P-labeled P8. (A) Timing of the reaction at 30°C. Prewarmed reaction components were mixed, and the reaction was stopped by boiling. The Mg^{2+}, ATP, and unlabeled dGTP concentrations were as in standard mixtures. (B) Effect of temperature on the formation of the P8-dGMP complex. (C) Effect of the addition of unlabeled dGTP. (D) Effect of the ATP concentration. (E) Effect of the $MgCl_2$ concentration.

TABLE 3. Protein P8-dGMP complex formation

Standard reaction	Relative yield (%)[a]
With [α-^{32}P]GTP	0
Minus phage	0
One-fifth the amount of phage	52
Minus extract	0
Extract without pLM3 but with pBR322	0
Extract from cells with pLM141	0
One-third the amount of pLM3 extract	31
Phage treated with 60 μg of trypsin per ml[b]	0
Phage treated with 300 U of nuclease per ml[c]	8
Extract treated with 300 U of nuclease per ml[d]	28
With 50 μg of actinomycin D per ml	32
With 20 μg of rifampin per ml[e]	100
With 30 μg of Triton X-100 per ml	104
With 450 μg of Triton X-100 per ml	79
With 3000 μg of Triton X-100 per ml	18
With 10% glycerol	108
With 25% glycerol	95
With phages PR3, PR4, PR5, L17, PR722, N, and I2-1[f]	75–100
With phage AP50 or φ29[f]	0

[a] The reaction sample was digested as described in the text and applied to a 15.5% polyacrylamide gel. The extent of the reaction was measured by densitometry. One hundred percent is about 3,000 cpm, which is equivalent to 0.1 pmol of deoxyguanosine. Since the amount of template is about 0.5 pmol of ends, the maximum yield is about 20% of the amount of template.

[b] The heat-disrupted phage was incubated with trypsin for 30 min at 37°C, followed by the addition of a 10-fold excess of trypsin inhibitor for 10 min at 37°C before addition to the reaction mixture. Trypsin inhibitor treatment alone did not have any effect on the reaction.

[c] The heat-disrupted phage was incubated with micrococcal nuclease for 30 min at 32°C, followed by 5 min at 65°C before addition to the reaction mixture.

[d] The cell extract with cloned genes was treated with nuclease as in footnote *c*.

[e] The rifampin was dissolved as described by Sinclair et al. (25).

[f] These phages, instead of PRD1, were tested one by one. The phage disruption was done as in the case of PRD1.

tion was specific for dGTP, since no detectable reaction occurred when GTP was used. Lowering both phage and extract concentrations reduced the yield, and the reaction was dependent on these components. Extracts from cells having only the pBR322 vector did not support any reaction, nor did extracts of cells containing pLM141, a plasmid that carries gene I but not gene VIII (15). Treatment of heat-disrupted phage with trypsin followed by trypsin inhibitor treatment before the addition of the phage to the reaction mixture led to a loss of activity. Nuclease treatment of the phage also led to a loss of activity. The addition of actinomycin D led to reduced activity, but rifampin did not have any effect. Low amounts of Triton X-100 and glycerol had slightly stimulatory effects, whereas higher concentrations reduced the activity. Closely related phages PR3, PR4, PR5, L17, PR722, N, and I2-1 all catalyzed the incorporation of [α-^{32}P]dGTP and no other nucleotide into protein P8. DNA from morphologically related lipid-containing phage AP50 infecting a gram-positive host did not stimulate the incorporation of any nucleotide into protein P8. The same was true for phage φ29, which actively forms a DNA-terminal protein-dAMP complex in *B. subtilis* (22).

When single-stranded DNAs from phages φX174 and fd were used in the assay, instead of disrupted phages, a very weak incorporation of all four deoxynucleotides to protein P8 was seen (data not shown).

Characterization of the bond between protein P8 and dGMP. The protein P8-dGMP complex was prepared with standard conditions and treated with nuclease. The nuclease-digested reaction mixture was passed through a Sephadex G-25 column. When acetate buffer with 0.1% SDS was used as an eluate, a shoulder of radioactivity was observed before the bulk radioactivity. Gel electrophoresis of the fractions showed the protein P8-dGMP complex in a peak of four fractions, with a slight tailing in subsequent fractions (data not shown). The early part of the peak showed very little low-molecular-weight radioactivity in the gel, whereas later fractions had strong low-molecular-weight radioactivity. No detectable protein P8-dGMP complex could be eluted from the column in the absence of SDS. The protein P8-dGMP complex from the early portion of the peak was concentrated, and the SDS was removed by acetone precipitation. The complex was hydrolyzed with HCl and analyzed for labeled phosphoamino acids by two-dimensional thin-layer electrophoresis. Figure 4 shows that the only labeled phosphoamino acid liberated from the P8-dGMP complex was phosphotyrosine. An unidentified labeled hydrolysis product was observed between the origin and phosphotyro-

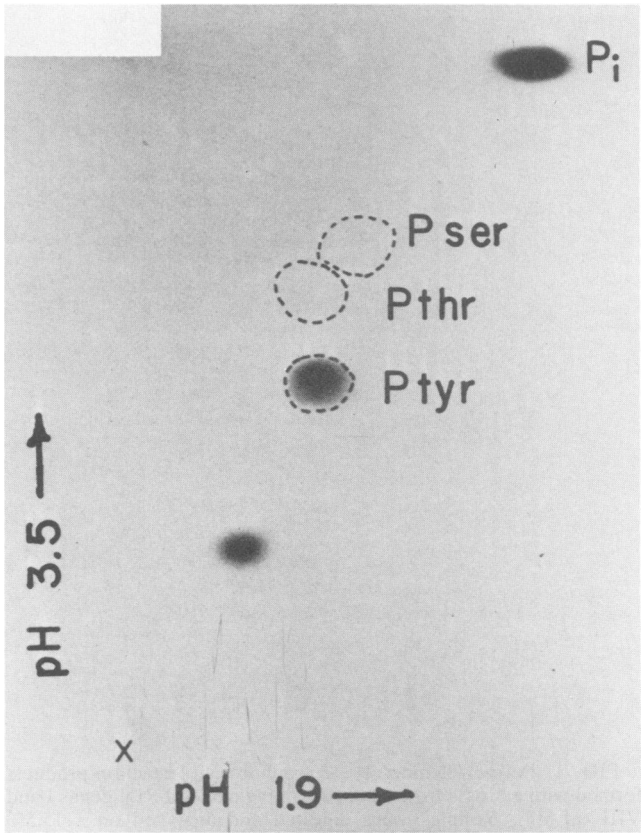

FIG. 4. Two-dimensional thin-layer electrophoresis of the acid-hydrolyzed (5.6 M HCl, 110°C, 135 min) P8–[α-^{32}P]dGMP complex. The dotted circles correspond to the ninhydrin-stained unlabeled phosphoamino acid standards on the autoradiogram of the thin-layer plate.

sine. This spot was strong after 90 min of hydrolysis but was diminished after 120 and 135 min, whereas the phosphotyrosine spot became more intense (data not shown). This indicated that this spot is a partial hydrolysis product of P8-dGMP containing labeled phosphotyrosine.

The acetone-precipitated P8-dGMP complex was sensitive to pronase (Fig. 5b) and to snake venom phosphodiesterase I (Fig. 5c), whereas it was resistant to spleen phosphodiesterase II and to alkaline phosphatase (Fig. 5d and e). Phosphodiesterase I recognizes a free 3'-OH group and cleaves the diesterase bond between the phosphorus and the next nucleotide or other group, whereas phosphodiesterase II needs a free 5'-OH group to make the diesterase bond cleavage. The resistance to alkaline phosphatase indicated that the labeled phosphate group is not free.

To study the possibility of a phosphoramidate, the P8-dGMP complex was incubated with 0.1 M HCl at 37°C for 2 h, a treatment hydrolyzing phosphoramidates (9). Compared with the untreated control, 96% of the p8-dGMP complex radioactivity, was left after this treatment.

Involvement of host replication functions in P8-dGMP complex formation and in phage replication. To test the necessity of host replication functions for the formation of the P8-dGMP complex, pLM3 containing early phage genes I and VIII with transferred to *E. coli* strains thermosensitive for replication genes *dnaB*, *dnaE*, *dnaA*, *dnaC*, *dnaG*, *dnaZ*, and *dnaP*. With extracts from these transformed cells, it was possible to form the P8-dGMP complex at a low temperature with all the mutants except *dnaZ* (Fig. 6j, top). When extracts were pretreated at 37°C for 20 min before being assayed at 30°C, all the other reactions behaved as though they were thermosensitive with the exception of that containing the primase mutation, *dnaG* (Fig. 6g). There were differences among different mutants in the degree of sensitivity in the same gene, as seen in the case of *dnaB* mutants (Fig. 6b, c, and d) and *dnaC* mutants (Fig. 6h and i).

PRD1 replication was tested in mutants defective in the helicase protein (*rep*) (24), in a strain in which the *dnaA* function was replaced by an integrated F-factor, and in a *dnaB* mutant which is dependent on P1 *ban* replication protein (23). Plasmid pLM2 was transferred to strains JW302, JW1073, and RS116 (Table 1). The phage plating efficiency was measured in these strains and was found to be 0.8 for *rep* [JW302(pLM2)], 0.7 for *dnaA* replaced by F-replication factor [JW1073(pLM2)], and 0.7 for *dnaB* replaced by P1 *ban* [RS116(pLM2)]. Plating of PRD1 was also tested in *S. typhimurium* strains carrying mutations in *supX* (21). The efficiency of plating was normal in several different point and deletion mutant strains, indicating that topoisomerase I coded for *supX* is not necessary for PRD1 replication.

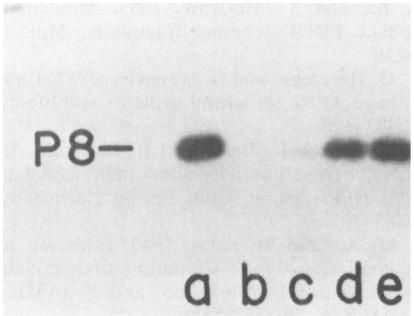

FIG. 5. Polyacrylamide gel electrophoresis after enzymatic hydrolysis of the P8–5'-[α-^{32}P]dGMP complex purified by column chromatography. Untreated (a). Digestion with: (b) 2 μg of pronase; (c) 0.03 U of snake venom phosphodiesterase I; (d) 0.035 U of bovine spleen phosphodiesterase II; (e) 2.5 U of bacterial alkaline phosphatase.

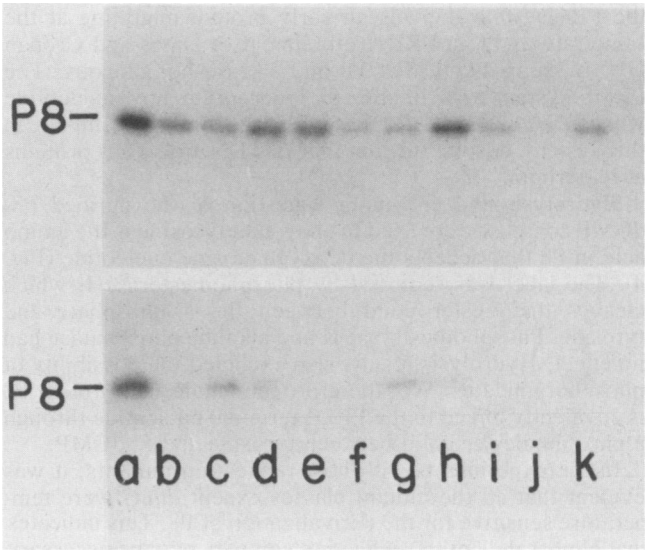

FIG. 6. Formation of the P8-dGMP complex with extracts from cells containing cloned PRD1 genes I and VIII and various *dna ts* mutations. Extracts were preincubated at 30 (top) or 37°C (bottom) for 20 min before being assayed at 30°C. Polyacrylamide gel electrophoresis of reaction products with extracts from cells bearing *ts* mutations is as follows: (a) wild-type cells; (b, c, and d) *dnaB* mutants; (e) *dnaE*; (f) *dnaA*; (g) *dnaG*; (h and i) *dnaC*; (j) *dnaZ*; (k) *dnaP*.

DISCUSSION

Early PRD1 genes I and VIII must be functional to form the initial replication complex between protein P8 and dGMP, as shown by mutant extract complementation data (Fig. 2). When cell extracts were prepared from cells containing these two genes on a plasmid (pLM3) (15), it was possible to increase the P8-dGMP complex formation by two orders of magnitude compared with infected-cell extracts. The increase in activity was probably due to the relatively large amount of underivatized P8 in cells containing plasmid pLM3, since there is no template available to catalyze the derivatization of P8. In infected cells, the P8 that is formed is probably used immediately for phage DNA synthesis. The plasmid-bearing extracts made it possible to further characterize this reaction and the structure of the complex. The reaction is dependent on template DNA with the terminal protein. The only phage proteins needed are P1 and P8 itself. The, albeit, weak reaction with all nucleotides and protein P8 when single-stranded ϕX174 and fd DNAs are used as a template indicates that protein P8 is capable of accepting any nucleotide and that specificity in selecting the nucleotide is not a property of this protein. This is similar to the finding made with adenovirus terminal protein (11). The system, however, is specific for deoxynucleotides, since GTP did not show any activity. Seven other closely related phages directed only dGMP incorporation, indicating that the 5'-terminal nucleotide is conserved in these phages. It is known that in

the PR4 system there is an early protein migrating at the same rate as P8 in PRD1 (protein 5A of Davis and Cronan [7]). It seems likely that P8 and 5A are homologous. The PRD1 system was unable to incorporate any nucleotide when DNA of *B. subtilis* phage ϕ29 was used as a template in this system, despite the fact that this DNA also has proteins at its termini.

Hydrolysis and enzymatic digestion of the purified P8-dGMP complex were used to show that tyrosine is the amino acid in P8 that accepts the deoxyguanosine nucleotide (Fig. 4). The label was removed by phosphodiesterase I, which cleaves the diester bond between the 5′-phosphate and tyrosine. Phosphodiesterase II and alkaline phosphatase had no effect. Hydrolysis results also excluded the possibility of phosphoramidates. We, therefore, conclude that protein P8 is covalently linked to the PRD1 terminal nucleotide through a phosphodiester bond between tyrosine and 5′-dGMP.

In the experiments with host replication mutants, it was evident that all the mutant classes except *dnaG* were temperature sensitive for the derivatization of P8. This indicates that almost the entire replication complex may be necessary for this initiation reaction. However, interpretation of temperature sensitivity of DNA synthesis must await further biochemical characterization. Neither the adenovirus (26) nor ϕ29 (28) system appears to require host polymerase for complex formation. The *rep* gene product (a helicase) is not important in PRD1 replication, nor is topoisomerase I. Davis and Cronan (7) reported that PR4 DNA synthesis is normal in the presence of nalidixic acid, which indicates that DNA gyrase (topoisomerase II) is also not necessary for PRD1 DNA replication.

The comparison with other DNA terminal proteins (adenovirus and ϕ29) shows that although each system is specific, there is not an overall restriction to a single phosphoamino acid or to a purine or pyrimidine base when the replication initiation complex is made. Thus, the adenovirus termini link deoxycytidine to serine, ϕ29 links deoxyadenosine to serine, and PRD1 links deoxyguanosine to tyrosine. The principle of a terminal DNA bound protein and an auxiliary early viral protein necessary for making the initial complex seems to occur in adenovirus (27), ϕ29 (14), and bacteriophage PRD1 (this paper).

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