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Bacteriophage φW-14-Infected *Pseudomonas acidovorans* Synthesizes Hydroxymethyldeoxyuridine Triphosphate

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The infection of *Pseudomonas acidovorans* with bacteriophage φW-14 leads to the gradual disappearance of dTTP from the cells and to the appearance of hydroxymethyl dUTP (hmdUTP). Infected-cell extracts contain dUMP hydroxymethylase and activities converting hmdUMP to hmdUDP and hmdUTP. Hydroxymethylase appears immediately after infection, reaching a maximum 20 min later. Thymidylate synthase activity decreases to less than 10% of the preinfection level during the initial 40 min after infection. Newly replicated DNA contains 2 to 3% hydroxymethyluracil. Although uracil is released from newly replicated DNA by acid hydrolysis, uracil is not incorporated as such into φW-14 DNA, and dUTP is not present in the acid-soluble pool of infected cells. It is concluded that the thymine and α-putrescinylthymine in φW-14 DNA are formed from hydroxymethyluracil at the polynucleotide level and that an intermediate in one or both of these conversions is degraded to uracil by acid hydrolysis. The modification of hydroxymethyluracil is coupled tightly to replication.

The hypermodified pyrimidine α-putrescinylthymine (putThy) replaces half of the thymine in the DNA of bacteriophage φW-14 (8; M. Mandel, personal communication). Thymine is not a precursor of putThy, but the two bases originate from a common precursor (7). Neither thymine nor putThy is synthesized at the mononucleotide level in infected cells of *Pseudomonas acidovorans* because infection leads to the appearance of dTTPase activity and the cells do not contain dTTP or putThy nucleotides when phage DNA is made (11). A new pyrimidine deoxyribonucleoside triphosphate appears in the acid-soluble pool of infected cells (11). In this paper we show that this new nucleotide is 5-hydroxymethyldeoxyuridine triphosphate (hmdUTP) and that newly replicated DNA contains hydroxymethyluracil (hmUra). We show also that infected cells contain a dUMP hydroxymethylase as well as kinase activities phosphorylating hmdUMP to hmdUDP and hmdUTP.

MATERIALS AND METHODS

Organisms, media, and growth conditions. Phage φW-14 was grown on *P. acidovorans* strain 29 at 30°C. The multiplicity of infection was always 10 at a cell density of 3×10^8 ml^{-1}. The following media were used: Casamino Acids-mannitol (CAAM) (8) for routine propagation of phage and the preparation of infected-cell extracts, Tris-Casamino Acids-succinate medium (TCS) (9) for uracil labeling of DNA, and a Tris minimal medium (4) with the phosphate content reduced to 0.3 mM and supplemented with 0.2% succinate and 0.2% Norite-treated Casamino Acids for the labeling of nucleotide pools.

Isolation and characterization of DNA. The labeling, extraction, and hydrolysis of replicating DNA were described previously (9, 11). The bases were separated by two-dimensional thin-layer chromatography on cellulose sheets (20 by 20 cm) using *t*-butanol-ethyl methyl ketone-concentrated HCl-water (40:30:10:20, vol/vol) (2) in the first dimension and *n*-butanol-water-concentrated NH_4OH (86:14:5, vol/vol) (5) in the second dimension. The buoyant density of the DNA was determined by isopycnic density-gradient centrifugation. A sample of the DNA was mixed with labeled phage DNA, and the density of the mixture was brought to 1.700 g ml^{-1} with saturated CsCl solution. After centrifugation for 60 h at 30,000 rpm in a Beckman SW50.1 rotor, fractions were collected from the bottom of the tube and assayed for radioactivity.

Labeling and extraction of acid-soluble nucleoside triphosphate pools. Exponential cultures were labeled for at least one generation before initiation of the experiment with either [2-^{14}C]uracil (5 μCi ml^{-1}; specific activity, 59 μCi μmol^{-1}) or [^{32}P]orthophosphate (specific activity, 50 μCi μmol^{-1}). The cultures were sampled at the desired times. For uracil labeling, 3-ml samples were filtered on 0.45-μm membranes (Millipore Corp.), and the membranes were extracted immediately in 2 ml of 0.3 M formic acid at 0°C for 30 min; the supernatants obtained by centrifugation were lyophilized and then dissolved in 200 μl of water. For ^{32}P labeling, 0.5-ml samples were added to 0.1 ml of 2 M formic acid at 0°C; after 30 min the extracts were centrifuged and the supernatants were retained for chromatography.

Three-dimensional separation of nucleoside

triphosphates. Extracts (50 to 100 μl), prepared as described above, were mixed with 10 nmol of each of the eight normally occurring nucleoside triphosphates plus dUTP and applied to polyethyleneimine-cellulose thin-layer chromatograms (20 by 20 cm). The chromatograms were washed in absolute methanol for 5 min and, after drying, developed in two dimensions as described previously (14). As shown in Fig. 1, the eight normally occurring nucleoside triphosphates are readily separated from each other in this system. However, dTTP, dUTP, and hmdUTP overlap each other. To resolve them, a strip of the chromatogram, containing the dTTP-region and running perpendicular to the second dimension towards the start line of the first dimension, was cut out (Fig. 1). The strip was washed in methanol for 5 min and dried, and a piece of Whatman 3MM filter paper was stapled to the end furthest from the dTTP spot. An excellent separation of dUTP and dTTP was obtained by 3 h of ascending chromatography in *n*-butanol–methanol–concentrated HCL–water (70:10:5:15, vol/vol) (6). After extensive drying and a methanol wash, the dUTP and dTTP markers were localized by UV light and the strips were placed overnight on X-ray film. Radioactive spots were cut out and counted in a Packard Tri-Carb scintillation counter.

Elution from chromatograms. Nucleotides were eluted from PEI-cellulose thin-layer chromatograms onto filter paper strips with 2 M NH_4OH (16). The compounds were eluted from the filter paper with water.

Identification of [2-^{14}C]hmdUrd nucleotides. The isolated compounds were eluted from the PEI-cellulose chromatograms as described above, and the eluates (30 to 50 μl) were dessicated overnight over $CaCl_2$. The dessicated samples were redissolved in 10 μl of 0.25 M Tris-chloride, pH 8.0, containing 20 nmol of dTTP, 10 nmol of dUTP, 10 nmol of 5-hydroxymethyl-deoxyuridine (hmdUrd), and 0.3 U of alkaline phosphatase, incubated for 60 min at room temperature, and then applied to cellulose thin-layer plates. Thymidine, deoxyuridine, and hmdUrd (R_f 0.57, 0.39, and 0.25, respectively) were resolved by chromatography with 86% aqueous *n*-butanol to 14 cm from the origin (Fig. 2).

Preparation of extracts. Cells were collected by centrifugation, washed once with 0.9 M NaCl and resuspended at 5×10^{10} ml^{-1} in 50 mM Tris-hydrochloride (pH 7.5)–10 mM in β-mercaptoethanol. The cells were broken by sonication at 0°C, and debris was removed by centrifugation at 20,000 × *g* for 20 min. In certain cases, these crude extracts were treated with 1/10 volume of 10% streptomycin sulfate, and the supernatants were dialyzed overnight against 50 mM Tris-hydrochloride (pH 7.5)–10 mM β-mercaptoethanol. Protein concentrations were determined by the phenol method (10) with bovine serum albumin as the standard.

Thymidylate synthase and dUMP hydroxymethylase assays. The reaction mixtures contained in 50 μl: 50 mM Tris-hydrochloride (pH 7.5), 25 mM $MgCl_2$, 100 mM β-mercaptoethanol, 0.8 mM EDTA; 0.7 mM *dl*-L-tetrahydrofolate; 1 mM dUMP; 2.4 mM [^{14}C]formaldehyde (specific activity, 2,750 cpm $nmol^{-1}$); and 0.01 to 0.2 U of enzyme. After 5, 12, and 20 min at 30°C, 15-μl samples were removed and the reaction was stopped by boiling for 1 min. A 10-μl amount of each sample was spotted on PEI-cellulose thin-layer sheets prespotted with 5 nmol of dUMP and dTMP at each start point. The chromatograms were washed for 5 min in methanol, dried, and developed with methanol to the start line followed by 1 M formic acid–0.25 M LiCl to 10 cm from the start line. In this solvent, dUMP and dTMP are not resolved, but dUMP runs slightly slower than dTMP, and hmdUMP comigrates with dUMP. The combined dUMP and dTMP spots were cut out and counted. Alternatively, the boiled samples were centrifuged to remove denatured proteins, and 10 μl of the supernatant was mixed with 5 nmol of dUMP and dTMP and spotted on cellulose thin-layer plates. The plates were developed in a solvent consisting of 0.1 M potassium phosphate, pH 6.8, to which was added 600 g of ammonium sulfate and 20 ml of *n*-propanol per liter (17). This solvent separates dUMP cleanly from dTMP, and hmdUMP comigrates with dUMP. By counting the dUMP–hmdUMP and the dTMP spots separately, the activities of thymidylate synthase and dUMP hydroxymethylase in the enzyme preparations can be determined simultaneously.

Preparation of [^{14}C]hmdUMP. The reaction mixture contained, in 1 ml: 50 mM Tris-hydrochloride, pH 7.5; 10 mM $MgCl_2$; 100 mM β-mercaptoethanol; 0.88 mM *dl*-L-tetrahydrofolate; 0.25 mM dUMP; 4.4 mM [^{14}C]formaldehyde (specific activity, 2,750 cpm/nmol); and 3 U of a partially purified preparation of φW-14-induced dUMP hydroxymethylase. After 60 min at 30°C, the reaction was stopped by the addition of formic acid to 2 M, followed by 1 ml of 20% Norite in water to absorb nucleotides. The Norite was washed once with 1 M formic acid and twice with water. Nucleotides were eluted from the Norite with 3 × 1 ml of 50% ethanol–50 mM ammonia, and the combined eluates were evaporated to dryness. The residue was dissolved in 200 μl of water and applied to a PEI-cellulose thin-layer sheet as a 2-cm-wide band. The chromatogram was washed with absolute methanol (5 min) and developed in methanol to the start followed by 1 M formic acid–0.25 M LiCl to 12 cm from the start. The UV-absorbing material, containing dUMP and [^{14}C]hmdUMP, was eluted with NH_4OH, dessicated, redissolved in 50 μl of water, and spotted on a cellulose thin-layer sheet. This chromatogram was developed to 15 cm from the origin in isopropanol–water–concentrated HCl (65:18.4:16.6, vol/vol) (1) to separate hmdUMP from dUMP. Autoradiography showed that all the radioactivity was present in a single spot. This spot was cut out, the radioactivity was eluted, and the material was identified as [^{14}C]hmdUMP by high-pressure liquid chromatography and by chromatography of the ^{14}C-labeled deoxyribonucleoside produced from it by alkaline phosphatase digestion. About 70 nmol of [^{14}C]hmdUMP was obtained with a specific activity of about 2,700 cpm/$nmol^{-1}$.

Phosphorylation of hmdUMP. Reaction mixtures contained in 10 μl: 100 mM Tris-hydrochloride, pH 7.5; 100 mM $MgCl_2$; 5 mM ATP; 0.6 mM [^{14}C]-hmdUMP (specific activity, 2,700 cpm $nmol^{-1}$); and 2 μl (40 μg of protein) of a crude extract obtained from cells harvested 40 min after infection. After 30 min at 22°C, portions were spotted on PEI-cellulose thin-

layer sheets prespotted with 5 nmol of dUMP, dUDP, and dUTP as markers. The chromatograms were washed for 5 min in absolute methanol, dried, and developed stepwise as follows: methanol to the start line, 2 M sodium formate (pH 3.4) to 2 cm above the start line, and 4 M sodium formate (pH 3.4) to 10 cm above the start line. The chromatograms were dried, washed for 5 min with methanol, and dried again. Radioactive materials were detected by radioautography. In the solvent system used, hmdUDP and hmdUTP comigrate with dUDP and dUTP, respectively.

Enzyme units. One unit of enzyme is the amount that catalyzes the conversion of 1 nmol of substrate per min. Specific activity is given as nanomoles per minute per milligram of protein.

Chemicals. Nucleosides, nucleotides, *dl*-L-tetrahydrofolic acid, and *Escherichia coli* alkaline phosphatase type III were from Sigma Chemical Co., St. Louis, Mo. The tetrahydrofolate was dissolved in 1 M Tris-hydrochloride (pH 7.5)–1 M β-mercaptoethanol and stored frozen under nitrogen. PEI-cellulose thin-layer sheets were prepared on plastic (15). Cellulose thin-layer sheets were of two kinds: on aluminium foil, from E. Merck AG, Darmstadt, West Germany; and on plastic, from Eastman Organic Chemicals, Rochester, N. Y. [^{32}P]orthophosphate was from Atomenergikommisiones Forsøgsanlaeg, Risø, Denmark; [^{14}C]-formaldehyde and [2-^{14}C]uracil were from the Radiochemical Centre, Amersham, England; [5-^{3}H]uracil and [6-^{3}H]uracil were from New England Nuclear, Montreal, Canada.

RESULTS

Identification of hmdUTP in infected cells. Infection of *P. acidovorans* with φW-14 results in the disappearance of dTTP from the acid-soluble nucleotide pool and the appearance of a new pyrimidine deoxynucleoside triphosphate which runs very close to dTTP in the conventional two-dimensional chromatographic system used for separating nucleoside triphosphates (14, 16).

Acid-soluble pools were extracted from [2-^{14}C]uracil-labeled cells just before and 35 min after infection. After the addition of unlabeled dUTP and dTTP, the extracts were subjected to three-dimensional thin-layer chromatography as described in Materials and Methods followed by radioautography. The uninfected extract contained a radioactive compound comigrating with the dTTP marker. The infected extract contained most of the label in a compound migrating slower than both the dTTP and dUTP markers in the third solvent (Fig. 1). The radioactive areas were excised from the chromatograms. The radioactive material was eluted, mixed with unlabeled dUTP, dTTP, and hmdUrd, digested with alkaline phosphatase, and re-chromatographed on cellulose thin-layer sheets. Autoradiography showed that the compound from the uninfected cells was converted to thymidine by this treatment, that from the infected cells to hmdUrd (Fig. 2). Thus, the unknown nucleoside triphosphate present in infected cells is hmdUTP.

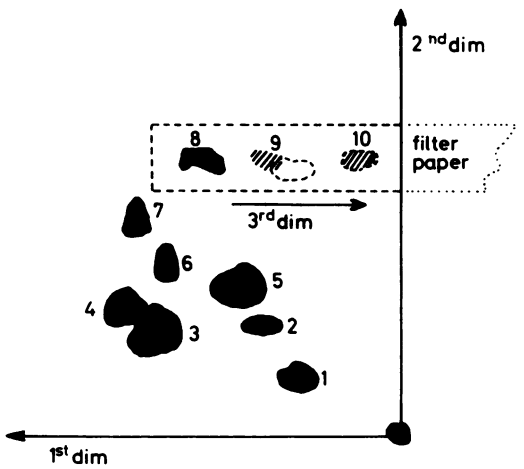

FIG. 1. *Three-dimensional separation of nucleoside triphosphates. The nucleoside triphosphates obtained from a [^{32}P]orthophosphate-labeled culture of P. acidovorans 25 min after infection with φW-14 were separated on PEI-cellulose thin-layer sheets as described in the text. Radioactive spots on the autoradiogram made after the second dimension are indicated by black spots. The strip subjected to additional chromatography (third dimension) is outlined by the broken line. Radioactive spots after third dimension are indicated by hatched areas. The spots are identified as follows: 1, GTP; 2, dGTP; 3, ATP; 4, CTP; 5, UTP; 6, dATP; 7, dCTP; 8, hmdUTP-dTTP (after second dimension); 9 and 10, hmdUTP and dTTP (after the third dimension), respectively. The broken circle refers to the unlabeled marker dUTP after the third dimension.*

Deoxyribonucleoside triphosphate pools after infection. The three-dimensional thin-layer technique was used to determine deoxyribonucleoside triphosphate pools as a function of time after infection. The dTTP pool starts to decrease 6 min after infection, and by 35 min, when the rate of phage DNA synthesis is maximal (7), the level of dTTP has decreased by more than 90% (Fig. 3). hmdUTP appears 10 min after infection, reaching a maximal level 10 min later (Fig. 3). The pools of dCTP, dGTP, and dATP decrease by about 50% during the first 10 min after infection and then remain constant (data not shown).

dUMP hydroxymethylase activity of infected cultures. Crude extracts made from cells harvested 40 min after infection catalyze the tetrahydrofolate-dependent transfer of [^{14}C]-formaldehyde to dUMP. However, the product of the reaction comigrates with dUMP rather than dTMP on cellulose thin-layer chromato-

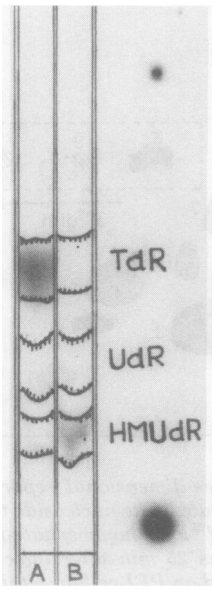

FIG. 2. *Autoradiogram of ^{14}C-labeled pyrimidine deoxyribonucleosides derived by alkaline phosphatase digestion of (A) dTTP from an uninfected culture of P. acidovorans and (B) the new pyrimidine deoxyribonucleoside triphosphate produced by φW-14-infected P. acidovorans. Cultures grown in the presence of [2-^{14}C]uracil were extracted, and the ^{14}C-labeled pyrimidine deoxyribonucleoside triphosphate was isolated and digested as described in the text. Unlabeled thymidine, deoxyuridine, and hmUra were added as markers. The chromatogram was developed in 86% aqueous n-butanol.*

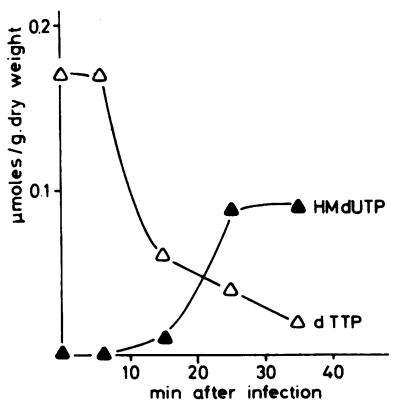

FIG. 3. *dTTP and hmdUTP pools in P. acidovorans as a function of time after infection with φW-14. The nucleotides were extracted and separated as described in the text.*

grams in the ammonium sulfate solvent (see Materials and Methods). After elution from the chromatograms, the product of the reaction was identified as [^{14}C]hmdUMP by (i) digestion with alkaline phosphatase followed by chromatography in the presence of appropriate deoxyribonucleoside markers (see Materials and Methods) and (ii) high-pressure liquid chromatography of the compound in the presence of authentic hmdUMP, dUMP, and dTMP (Lichrosorb C-18 eluted with 5 mM tetrabutylamine bisulfate–5 mM phosphate, pH 7.0).

dUMP hydroxymethylase appears very soon after infection, increasing to a maximal level by 20 min, when phage DNA synthesis starts (Fig. 4). Thymidylate synthase activity decreases steadily during the first 40 min after infection (Fig. 4).

hmdUMP kinase activity in infected-cell extracts. [^{14}C]hmdUMP was prepared as described in Materials and Methods. When incubated with an infected-cell extract, the [^{14}C]-hmdUMP was converted to hmdUDP and hmdUTP (Table 1).

hmUra is present in replicating DNA. Replicating DNA was pulse-labeled for 10 s with [6-^{3}H]uracil at 40 min after infection. The DNA was extracted and purified and then converted to the free bases by acid hydrolysis. The bases were separated by two-dimensional thin-layer chromatography in the presence of authentic

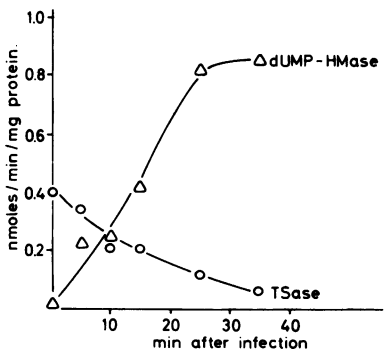

FIG. 4. *Specific activity of thymidylate synthase (TSase) and dUMP hydroxymethylase (dUMP-HMase) in streptomycin sulfate-treated extracts of φW-14-infected P. acidovorans as a function of time after infection. Extracts were prepared and assayed as described in the text.*

TABLE 1. *Phosphorylation of hmdUMP by crude extracts of φW-14-infected P. acidovorans*[a]

Reaction mixture	Amt of nucleotide (% of total)		
	hmdUMP	hmdUDP	hmdUTP
Complete	20	51	29
−ATP	87	8	5

[a] The reaction mixtures contained 0.6 mM [^{14}C]-hmdUMP and 5 mM ATP. The amounts of hmdUMP, hmdUDP, and hmdUTP present after 30 min of incubation at 22°C were determined by thin-layer chromatography.

standards. Some 2 to 3% of the radioactivity recovered from the DNA was in hmUra (Table 2). Thymine, uracil, and putThy were also labeled; the source of the uracil will be discussed later. Chasing pulse-labeled cells with 100-fold excess of unlabeled uracil did not stop incorporation very quickly: the chased cells contained 5 to 10 times more label in DNA than did the pulsed cells (data not shown). The DNA from cells labeled continuously from 20 to 40 min after infection contained little, if any, labeled hmUra (Table 2). These results show that although replicating DNA contains hmUra, the amount is quite small. This was confirmed by measuring the buoyant density of the replicating DNA (Fig. 5). The putThy and thymine residues contribute to the unusually low buoyant density of φW-14 DNA (8). Therefore, unmodified replicating DNA should be denser than vegetative φW-14 DNA. The DNA labeled by a 30-s pulse of [6-^{3}H]uracil was of the same density as phage DNA, but some 30% of the DNA labeled by a 10-s pulse was denser than phage DNA (Fig. 5). The denser DNA was absent from cells chased with unlabeled uracil after the 10-s pulse (Fig. 5).

Uracil is not incorporated into replicating DNA. Pulse-labeled DNA appeared to contain a significant amount of uracil (Table 2). Although cytosine can be deaminated to uracil during acid hydrolysis, this is not the source of the uracil in replicating DNA. When infected cells were pulse-labeled with [5-^{3}H]uracil, there was no labeled uracil released from the DNA by acid hydrolysis (Table 2). The label in putThy and thymine was assumed to come from contaminating [6-^{3}H]uracil in the [5-^{3}H]uracil. Furthermore, when the nucleotide pools of infected cells were labeled with [5-^{3}H]uracil, labeled dUTP was not detected in the acid-soluble extracts (Table 3). Although, as pointed out previously, dTTP, dUTP, and hmdUTP overlap somewhat in the usual two-dimensional system used to separate the nucleoside triphosphates, only

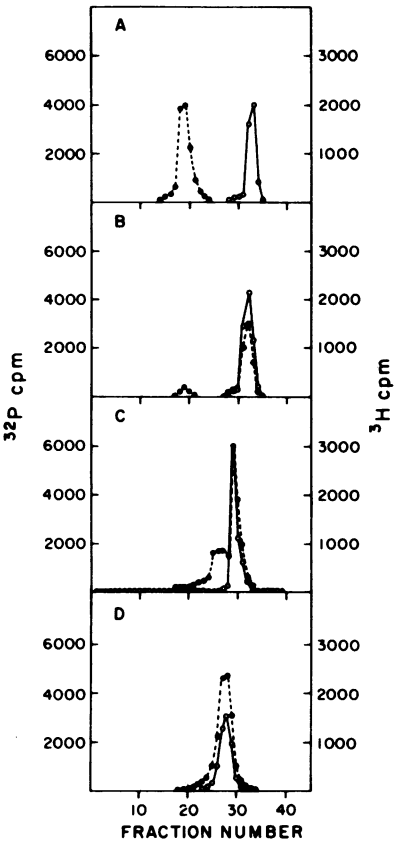

FIG. 5. *Isopycnic density gradient centrifugation of newly replicated DNA from P. acidovorans. The DNA was pulse-labeled with [6-^{3}H]uracil, chased with excess unlabeled uracil, extracted, and processed as described in the text. (A) Uninfected cells, 30-s pulse. (B) Infected cells, 30-s pulse. (C) Infected cells, 10-s pulse. (D) Infected cells, 10-s pulse followed by chase. Symbols: ●, newly replicated, [6-^{3}H]uracil-labeled DNA; ○, ^{32}P-labeled phage DNA.*

TABLE 2. *Base composition of replicating φW-14 DNA*[a]

Base	Labeled precursor		
	[6-^{3}H]uracil (10-s pulse)	[5-^{3}H]uracil (10-s pulse)	[6-^{3}H]uracil (20-min labeling)
putThy	2,200[b] (7.7)	531 (1.7)	14,430 (18.2)
Cytosine	15,230 (53.2)	27,024 (86.5)	34,585 (43.6)
hmUra	740 (2.6)	26 (>0.1)	157 (0.2)
Uracil	1,830 (6.4)	28 (>0.1)	180 (0.2)
Thymine	5,205 (18.2)	980 (3.1)	21,976 (27.7)
Unhydrolyzed nucleotides	3,410 (11.9)	2,653 (8.5)	7,987 (10.0)

[a] DNA was labeled, extracted, and hydrolyzed as described in the text. The bases were separated by two-dimensional thin-layer chromatography on cellulose sheets.

[b] Counts per minute in the area cut from the chromatogram. The figures in parentheses represent the percentage of recovered radioactivity. Total recovery of applied radioactivity was >90%.

TABLE 3. *[5-^{3}H]uracil labeling of nucleotide pools*[a]

Nucleotide	Uninfected	Infected
UTP	7,205[b]	8,560[b]
CTP	13,700	28,262
dCTP	4,293	2,233
dUTP-dTTP-hmdUTP	41	25

[a] Nucleotide pools were labeled with [5-^{3}H]uracil, extracted, and analyzed as described in the text.

[b] Counts per minute in the area cut from the chromatogram after elution with *M* NH_4OH and the addition of Bray solution.

dUTP would be labeled by [5-^{3}H]uracil. This result confirms also the identification of the new deoxynucleoside triphosphate appearing after infection as hmdUTP.

DISCUSSION

The results reported here support and extend the conclusion (11) that the thymine and putThy residues in ϕW-14 DNA are synthesized from hmUra at the polynucleotide level. The synthesis of hmdUMP from dUMP and N_5,N^{10}-methylenetetrahydrofolate does not result in the oxidation of tetrahydrofolate to dihydrofolate. This explains why extracts prepared after infection of a thymidine auxotroph of *P. acidovorans* do not oxidize tetrahydrofolate in the presence of formaldehyde and dUMP (7). At 20 min after infection, significant thymidylate synthase activity is still present in extracts prepared from infected wild-type cells (Fig. 4), and such extracts still oxidize tetrahydrofolate in the presence of formaldehyde and dUMP (7).

The drop in the level of dTTP in the infected cell is a consequence of the appearance of dTTpase activity (11) and of the inhibition of host thymidylate synthase (Fig. 4). However, there is still detectable dTTP in the cells 10 to 15 min after infection when host DNA synthesis has stopped. This suggests that there may be a direct inhibition of host DNA polymerase after infection, since host DNA is not degraded to acid-soluble fragments (11). When phage DNA synthesis reaches its maximal rate some 30 min after infection (7), there is little dTTP left in the cells. The phage-induced dTTPase may be sufficient to prevent the incorporation of thymidine into phage DNA. However, in T4-infected *E. coli*, a multienzyme complex appears to synthesize the pyrimidine nucleotide precursors for T4 DNA and to incorporate them into the DNA (19). This complex may discriminate against exogenous deoxynucleotides. A similar complex in ϕW-14-infected cells would also be a factor in discriminating against exogenous thymidine.

Since uracil is not incorporated directly into ϕW-14 DNA, hmUra must be the precursor of both thymine and putThy. Thymine is not a precursor of putThy at the polynucleotide level because the thymine methyl and the methylene group at C-5 of putThy are formed from methylenetetrahydrofolate at the same oxidation level (7). It is not clear whether hydroxymethyluracil goes to thymine and putThy by completely divergent routes or whether there is a common intermediate beyond hmUra. In pulse-labeled DNA, more label is found in thymine than in putThy (Table 2), and the uracil released may come from an unstable intermediate between hmUra and putThy. hmUra is stable under the hydrolysis conditions used (2).

The biosynthesis of ϕW-14 and SP10 DNA have much in common. In SP10 DNA, 15 to 20% of the thymine is replaced with a base carrying a secondary amine function at C-5 of the uracil ring (M. Mandel, personal communication). The thymine is formed at the polynucleotide level, probably from hmUra (3, 18). Infection with SP10 also leads to the inhibition of host thymidylate synthase (18) and to the appearance of dTTPase (12) and dUMP hydroxymethylase (18) activities; hmdUTP is also found in the infected cells (3). It is of considerable interest that the two phages, one attacking a gram-negative host and the other attacking a gram-positive host, should be so similar in their biochemistry.

Modification of the hmUra residues in ϕW-14 DNA appears to be coupled tightly to replication, since very short pulses of radioactive uracil are required to reveal any marked density heterogeneity in the replicating DNA. Modification is also coupled tightly to replication in SP10-infected *Bacillus subtilis* (H. Witmer, personal communication). Glucosylation of T4 DNA may be coupled to replication (13).

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