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Enzymatic Degradation of Uracil-Containing DNA

II. Evidence for *N*-Glycosidase and Nuclease Activities in Unfractionated Extracts of *Bacillus subtilis*

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Further studies have confirmed our earlier observation that in the presence of EDTA, degradation of phage PBS2 [^{3}H]uracil-labeled DNA is effected by an *N*-glycosidase activity in extracts of *Bacillus subtilis* that removes free uracil from DNA. In addition, such extracts contain a nuclease activity that attacks PBS2 DNA in the presence of $CaCl_2$. The nuclease activity is not observed under conditions that inactivate *N*-glycosidase activity but does attack DNA that has been preincubated to remove uracil by *N*-glycosidase action. We therefore postulate that the nuclease requires *N*-glycosidase action to generate substrate for its activity, i.e., the nuclease appears to attack depyrimidinated sites rather than uracil sites in phage PBS2 DNA.

Extracts from a variety of microbial sources have been shown to contain enzyme activity that specifically attacks uracil-containing DNA. W. Carrier and R. B. Setlow (Fed. Proc. 33:1599, 1974) briefly reported the degradation of phage PBS2 DNA labeled with [^{3}H]uridine by extracts of *Micrococcus luteus* and suggested that this was the result of nuclease activity. Subsequently, Lindahl (6) reported that in the presence of EDTA, crude extracts of *Escherichia coli* degraded labeled poly(dC,dU) as well as [^{3}H]cytosine-labeled DNA in which a fraction of the cytosine residues had been deaminated to uracil. He observed that the sole labeled product of reaction with these substrates was uracil, and designated this enzyme activity an *N*-glycosidase.

Recently our laboratory reported that in the presence of EDTA, phage PBS2 [^{3}H]uracil-labeled DNA is specifically degraded by extracts of either *E. coli* or *Bacillus subtilis* (2). Under conditions that preclude the action of phosphorylases, the sole labeled product of reaction detected was free uracil. We therefore concurred with Lindahl (6) that in the presence of EDTA, degradation of uracil-containing DNA is effected by *N*-glycosidase activity. Finally, we reported that in contrast to the results obtained with extracts of uninfected *B. subtilis*, extracts of *B. subtilis* infected with phage PBS2 did not effect the release of uracil, and in fact, inhibited the activity present in extracts of uninfected cells (2).

Tomita and Takahashi (10, 11) have reported the results of independent studies on the degradation of ^{32}P-labeled PBS1 DNA by extracts of uninfected and phage-infected *B. subtilis*. They concluded that extracts of uninfected cells contain a nuclease activity that requires Ca^{2+} and that specifically attacks native or denatured PBS1 DNA. These workers also reported loss of the nuclease activity in extracts of phage PBS1-infected cells.

N-glycosidase and DNase activities can be readily confused, since both effect the release of acid-soluble labeled product from polydeoxyribonucleotide. The above-mentioned reports have not always clearly distinguished between these two activities, and in our opinion, considerable confusion exists concerning the true nature of the enzyme(s) that specifically attack uracil-containing DNA. In the present studies we show that unfractionated extracts of *B. subtilis* contain both *N*-glycosidase and nuclease activities and that both activities are lost in extracts of phage PBS2-infected cells.

MATERIALS AND METHODS

Bacterial stocks. *B. subtilis* SB5 was obtained from A. T. Ganesan, Stanford University. *E. coli* W3110 is from our own collection. All bacterial stocks were maintained on agar plates containing brain heart infusion broth (Difco).

Bacteriophage. Phage PBS2 was originally obtained from Alan Price, University of Michigan. Preparation of phage stocks and their storage was as described previously (2).

Isotopically labeled DNA. [5-^{3}H]uridine-labeled PBS phage were prepared using the modified procedure of Hunter et al. (4) previously described (2). Phage were harvested and purified by cesium chlo-

ride equilibrium sedimentation as described previously (2). For the preparation of double-labeled phage PBS2 DNA, in addition to 5 mCi of [5-^{3}H]uridine, 10 mCi of ^{32}P was added 5 min after infection of cultures with phage. The specific activity of the PBS2 [^{3}H]DNA varied between 50,000 and 150,000 cpm/μg. That of the double-labeled DNA was 65,000 to 140,000 cpm of ^{3}H per μg and 35,000 to 52,000 cpm of ^{32}P per μg. [^{3}H]thymidine-labeled *E. coli* DNA was prepared by the procedure of Thomas and Abelson (9).

Preparation of [^{3}H]polydeoxyuridine. The reaction mixture (1.0 ml) contained 0.2 M sodium-cacodylate buffer, pH 7.2, 1.0 mM β-mercaptoethanol, 1.0 mM $CaCl_2$, 60 nmol [^{3}H]dUTP; 1.0 mmol unlabeled dUTP, 750 nmol dT [as oligo$(dT)_{12-18}$] and terminal transferase (126,000 U/ml; 6.9 mg/ml). Enzyme (1,260 U) was added to initiate the reaction and a further 1,260 U was added at three 1-h intervals. After 3 h, more β-mercaptoethanol (1.0 mM) was added and also bovine serum albumin (1 mg/ml, final concentration). Incubation was at 37°C for 17 h, after which time it was determined that 60% of the [^{3}H]dUTP was in a form that was bound to DEAE-paper, i.e., polymerized. The incubation mixture was boiled for 5 min and centrifuged at 10,000 rpm for 10 min at 4°C to pellet precipitated protein. The supernatant was lyophilized and resuspended in 0.5 ml of distilled water. This was loaded onto a G-25 (medium) Sephadex column (60 by 1.5 cm) and eluted with water. Fractions (2.0 ml) containing acid-precipitable radioactivity were pooled, relyophilized, and rechromatographed by gel filtration. A single peak of radioactivity was eluted. Fractions containing >85% acid-precipitable radioactivity were pooled and stored in 10% ethanol.

Cell-free extracts. Cells were grown and phage infection was carried out at 37°C. Crude extracts of both uninfected and phage-infected cells were prepared as previously described.

Enzyme assays. (i) *N*-glycosidase activity. The standard incubation mixtures (0.13 to 0.25 ml) contained the following. Either single- or double-labeled PBS2 DNA (0.75 to 2.25 nmol as nucleotide) or labeled poly(dU) (0.2 to 0.39 nmol as nucleotide) was used as substrate. In addition, mixtures contained EDTA, 1.0 mM; bovine serum albumin, 1 mg/ml; sodium chloride, 20 to 25 mM; and crude extract dialyzed against 10.0 mM Tris-hydrochloride buffer, pH 8.0. All dilutions of extract were made into 10.0 mm Tris-hydrochloride, pH 8.0, with 0.1% bovine serum albumin. Incubations were for varying times at 37°C and were terminated as described previously (2). Radioactivity (^{3}H) in the acid-soluble fraction was determined by counting a portion of each sample in 7.0 ml of Aquasol or 10.0 ml of a mixture of Triton X-100 and toluene-based liquid scintillation fluid (1:2) in a liquid scintillation spectrometer. We have previously shown that all acid-soluble ^{3}H released under these conditions is in free uracil (2).

(ii) Nuclease activity. Nuclease activity was assayed with either double-labeled PBS2 DNA or *E. coli* ^{3}HDNA. The incubation mixtures (0.15 to 0.25 ml) contained PBS2 [^{3}H-^{32}P]DNA (0.75 to 2.25 nmol as nucleotide) or ^{3}H-labeled *E. coli* DNA, 3.27 nmol as nucleotide (specific activity, 9,333 cpm/nmol), all of the other components described for assay of *N*-glycosidase activity and, in addition, $CaCl_2$ (6 mM final concentration after taking into account the stoichiometry of EDTA chelation). In some instances, 5 mM $CaCl_2$ was added without EDTA in the incubation mixtures. Reactions were terminated, and radioactivity was measured as described above. Nuclease activity was determined by the amount of acid-soluble radioactivity (^{32}P from PBS2 DNA and ^{3}H from *E. coli* DNA) released.

Thin-layer and paper chromatography. Thin-layer chromatography on silica gel thin layers (sil G/UV) was carried out as previously described using the top phase of a mixture of ethylacetate-*n*-propanol-water (4:1:2) (2). Paper chromatography was carried out using Whatman 3MM paper and isobutyric acid (66%), EDTA (1 mM), and concentrated ammonium hydroxide (1%) as the solvent (7).

Materials. [5-^{3}H]uridine, [5-^{3}H]deoxyuridine, [5-^{3}H]uracil, [*methyl*-^{3}H]thymidine, and [5-^{3}H]deoxyuridinemonophosphate were purchased from Schwarz/Mann. ^{32}P was purchased from New England Nuclear Corp. and from Schwarz/Mann. [^{3}H]-deoxyuridinetriphosphate was obtained from the Radiochemical Center. Unlabeled bases, deoxynucleosides, and deoxynucleosidetriphosphate were obtained from Calbiochem and Sigma Chemical Co. Oligo $d(pT)_{12-18}$ was purchased from Collaborative Research. Terminal transferase was a gift from David Korn. The enzyme was purified by R. L. Ratloff of Los Alamos Laboratories, by the procedure of Chang and Bollum (1) and was stored frozen in 0.3 M potassium phosphate buffer at pH 7.0.

RESULTS

To designate an enzyme activity as an *N*-glycosidase, the sole product of its reaction with DNA must be a free base. In view of the fact that under their experimental conditions Tomita and Takahashi failed to observe free uracil, but did report the presence of deoxyuridine as a product of reaction with PBS1 DNA (11), we decided to rigorously reinvestigate the nature of the labeled product present in the acid-soluble fraction under our incubation conditions. These conditions exclude divalent cations by the use of EDTA and preclude nucleotide phosphorylase activity by eliminating inorganic phosphate from the reaction mixture.

We had previously shown by thin-layer chromatography that all of the labeled acid-soluble product from a reaction with [^{3}H]uridine-labeled PBS2 DNA migrates with the R_f of authentic unlabeled uracil (2). In the present experiments, comparison of the relative R_f of the acid-soluble radioactivity with that of [^{3}H]deoxyuridine and of [^{3}H]uracil identifies the labeled reaction product from both PBS2 DNA and from [^{3}H]poly(dU) as uracil (Fig. 1A and B). The result with the latter substrate established that single-stranded synthetic polymer containing only uracil is a substrate for the

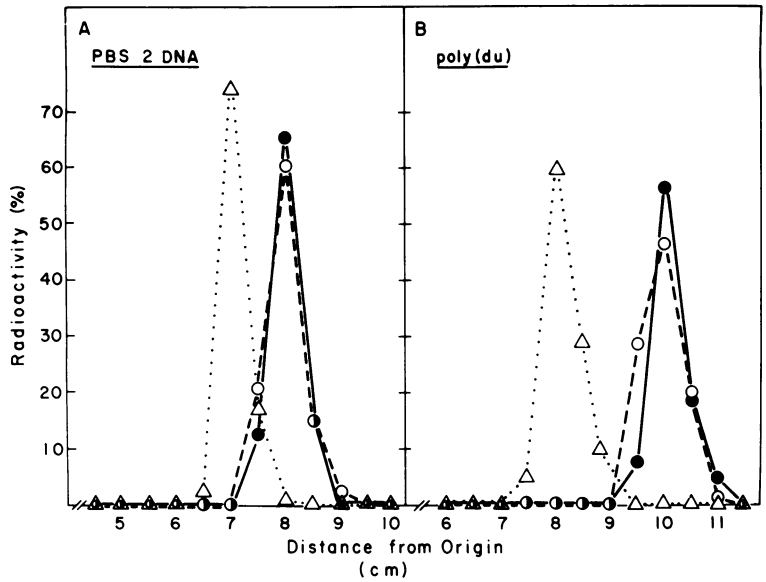

FIG. 1. *Thin-layer chromatographic analysis of the acid-soluble products in incubation of phage PBS2 DNA or poly(dU). Incubation mixtures (0.75 ml) contained 7.5 nmol (as nucleotide) of double-labeled PBS2 DNA (50,000 cpm of ^{3}H per nmol and 17,300 cpm of ^{32}P per nmol) or 0.39 nmol (as nucleotide) of [^{3}H]poly(dU) (174,000 cpm/nmol); 1.0 mM EDTA; 1 mg of bovine serum albumin per ml; 10 mM Tris-hydrochloride buffer, pH 8.0, and 0.75 mg of protein from a dialyzed crude extract of B. subtilis SB5. Reactions were terminated as previously described (2), and the acid-soluble fractions were saved. The acid-soluble fractions were ether-extracted three times and concentrated by evaporation. Portions (5.0 μl) containing 1,250 cpm of ^{3}H (A) and 1,435 cpm of ^{3}H (B) were applied to the origin of Brinkmann silica gel thin-layer plates (20 by 20 cm). [^{3}H]dUrd and [^{3}H]uridine were used as chromatographic markers. Chromatograms were developed by ascending chromatography using the solvent system described in Materials and Methods. Symbols: (△) [^{3}H]dUrd; (●) [^{3}H]uracil; (○) [^{3}H] in acid-soluble fraction.*

activity in *B. subtilis*. We have also carried out paper chromatography with a different solvent system (7) on the acid-soluble products of reaction with PBS2 DNA. Figure 2 shows a single peak of radioactivity in the region where unlabeled authentic uracil migrates. Reference [^{3}H]deoxyuridine migrates with the cold deoxyuridine standard and is clearly resolved from the labeled uracil product. Further experiments have confirmed our previous observations (2) that neither [^{3}H]dUMP nor [^{3}H]dUrd are converted to free uracil under our incubation conditions.

The *N*-glycosidase activity in crude extracts of *B. subtilis* is stimulated by salt. The addition of either NaCl or KCl causes stimulation of the degradation of [^{3}H]poly(dU) at an optimal concentration of about 20 mM. Similar results were obtained with labeled PBS2 DNA as substrate. Analysis of the acid-soluble fraction after incubation with 20 mM salt revealed that the product of incubation is still exclusively uracil.

To investigate DNase activity on PBS2 DNA under the aforementioned incubation conditions (1.0 mM EDTA and 20 mM salt), we incubated crude extracts of *B. subtilis* with DNA labeled with both ^{3}H and ^{32}P. As indicated in experiment 1 of Table 1, when 36.5% of the uracil in PBS2 DNA became acid soluble, all ^{32}P was still acid precipitable. The specificity of this reaction is indicated by the demonstration that the *N*-glycosidase is inhibited by an extract of phage PBS2-infected cells, as previously reported (2). In other experiments we observed release of as much as 85% of the labeled uracil without any significant ^{32}P release. (We have reproducibly observed that 15 to 25% of the radioactivity in PBS2 DNA labeled with [^{3}H]uridine is resistant to *N*-glycosidase action. It is highly likely that this fraction of the radioactivity is in cytosine residues in DNA.) This result rules out the presence of significant degradation of the DNA by nucleases. Endonucleolytic incisions in PBS2 DNA cannot be eliminated directly by these experiments. However, if present, phosphodiester bond breaks would have to be limited, since uracil constitutes 33% of the bases in PBS2 DNA, and endonucleolytic attack at all available uracil sites would render a significant fraction of the ^{32}P acid soluble.

Tomita and Takahashi (10, 11) reported that

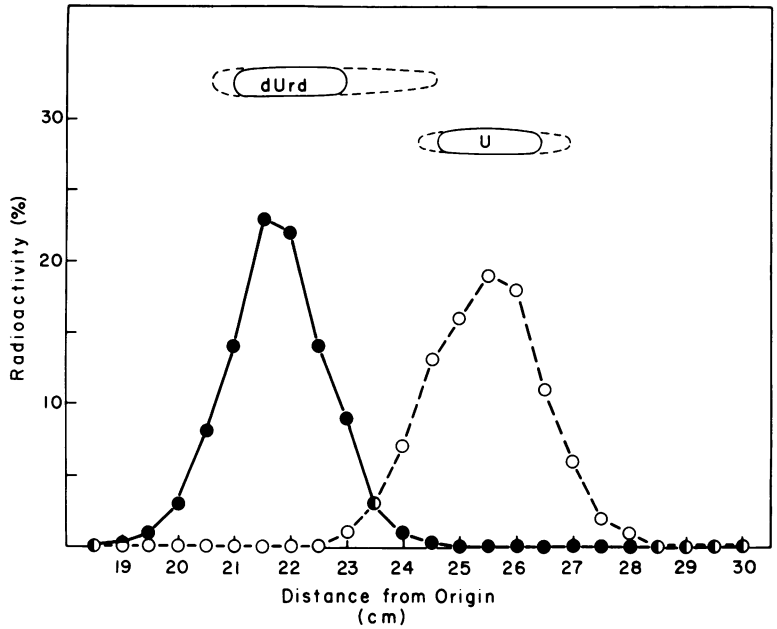

FIG. 2. *Paper chromatographic analysis of the acid-soluble products of incubation of phage PBS2 DNA. Incubation conditions were as described in the legend to Fig. 1A. A 2.0-μl portion of the concentrated acid-soluble fraction containing 20,000 cpm of ^3H was applied to the origin of a sheet of Whatman 3MM chromatography paper. The chromatogram was developed by descending chromatography for 20 h using the solvent system detailed under Materials and Methods. Unlabeled uracil and deoxyuridine and [^3H]deoxyuridine were used as chromatographic markers. Symbols: (●) [^3H]deoxyuridine marker; (○) ^3H in acid-soluble fraction.*

the nuclease activity that attacks native PBS1 DNA requires Ca^{2+}. Experiment 2 of Table 1 shows the results of incubating double-labeled PBS2 DNA with crude extracts of *B. subtilis* in the presence of Ca^{2+}. Under these conditions, there is increased ^3H release and a significant fraction of ^{32}P becomes acid soluble, indicating the presence of nuclease activity. The release of both isotopes into an acid-soluble form is inhibited in the presence of an extract of phage-infected cells (experiment 2, Table 1).

Chromatographic analysis of the acid-soluble fraction of a reaction carried out in the presence of $CaCl_2$ showed that all ^{32}P and about 20% of the ^3H remained at the origin, whereas the remainder of the ^3H migrated with the R_f of free uracil (Fig. 3). In contrast to the report of Tomita and Takahashi (11), we have not observed deoxyuridine as a product of reaction. We conclude that the *N*-glycosidase retains activity in the presence of $CaCl_2$, but in addition, in the presence of the divalent cation, DNase activity is observed, which results in significant degradation of the DNA.

Under conditions that result in complete degradation of PBS2 [^{32}P]DNA to acid-soluble products, only 10 to 15% of an equivalent concentration of *E. coli* DNA is degraded. Furthermore, the latter reaction is not inhibited by addition of extract from phage-infected cells. This result is consistent with the notion that the nuclease is specific for uracil-containing DNA. However, since we are not able to identify conditions under which nuclease activity can be observed without *N*-glycosidase activity, these results are equally consistent with the idea that the specific substrates for the nuclease are depyrimidinated sites created by *N*-glycosidase activity. Such a hypothesis would not require that the nuclease be inhibitable by a factor in phage infected extracts; direct inhibition of the *N*-glycosidase would preclude any nuclease activity. A number of experimental results support this contention.

(i) As shown in Fig. 4, the kinetics of inhibition of *N*-glycosidase and nuclease activities in the same extract of phage-infected cells are very similar. In our hands, extracts of phage-infected cells prepared 4 min after infection do not effect the release of either [^3H]uracil or ^{32}P from double-labeled PBS2 DNA.

(ii) Both *N*-glycosidase and nuclease activities are present in crude extracts of *E. coli*. Figure 5 compares the relative sensitivities of the two enzyme activities from *E. coli* and *B. subtilis* to heat inactivation and to treatment

TABLE 1. *Demonstration of nuclease activity in the presence of $CaCl_2$*[a]

Incubation conditions	% DNA degradation to acid-soluble product	
	^3H	^{32}P
Expt 1		
Extract of uninfected cells + EDTA (1.0 mM)	36.5	0
Extract of uninfected cells + extract of phage-infected cells + EDTA (1.0 mM)	0.5	0.3
Expt 2		
Extract of uninfected cells + $CaCl_2$ (2.0 mM)	69.8	42.4
Extract of uninfected cells + $CaCl_2$ (6.0 mM)	87.4	55.3
Extract of uninfected cells + extract of phage-infected cells + $CaCl_2$ (2.0 mM)	1.3	0.3
Extract of uninfected cells + extract of phage-infected cells + $CaCl_2$ (6.0 mM)	1.3	0.6

[a] Incubations (0.2 ml) contained double-labeled PBS2 DNA, 1.17 nmol (as nucleotide); EDTA, 1 mM; NaCl, 25 mM; Tris-hydrochloride buffer, pH 8.0, 10 mM; 1.0 mg bovine serum albumin per ml and 0.8 mg of protein from *B. subtilis* SB5 extract previously dialyzed against 10 mM Tris-hydrochloride, pH 8.0. In reactions in which $CaCl_2$ was present, it was assumed that each molecule of EDTA would chelate one atom of Ca, and the concentrations quoted reflect the calculated levels of free $CaCl_2$. For incubations that included extract of phage-infected cells, 0.2 mg of protein was added and preincubated with all the components of the incubation mixture, except substrate, for 5 min at 37°C. All reactions were initiated by the addition of substrate and were at 37°C for 30 min.

with *p*-chloromercuriphenylsulfonic acid. The data indicate that in general the formation of acid-soluble ^{32}P disappears in parallel with the loss of *N*-glycosidase activity. In no case is nuclease activity present in the absence of *N*-glycosidase.

(iii) The factor in extracts of phage-infected cells that causes inhibition of *N*-glycosidase activity in extracts of uninfected *B. subtilis* is insensitive to boiling for 10 min. Boiled extract also causes inhibition of ^{32}P release in extracts of uninfected cells (J. Duncan and E. Friedberg, unpublished observations).

A more direct proof of the hypothesis that depyrimidinated sites are the substrate for nuclease attack of PBS2 DNA is provided by the results of the experiment shown in Table 2. Double-labeled PBS2 DNA was preincubated in EDTA with an extract of *B. subtilis*, resulting in the loss of 75% of the ^3H and none of the ^{32}P. After phenol extraction, the DNA was reincubated with enzyme in the presence of $CaCl_2$. The release of ^{32}P into the acid-soluble phase was now effected by extracts of both uninfected and phage-infected cells, i.e., nuclease activity was no longer "inhibitable." In control experiments with phenol-extracted DNA not preincubated with enzyme, no release of ^{32}P was effected by extracts of phage-infected cells (Table 2).

TABLE 2. *Nuclease activity on depyrimidinated PBS2 DNA*[a]

Incubation conditions	Substrate			
	Native PBS2 DNA		Depyrimidinated PBS2 DNA	
	^3H released (%)	^{32}P released (%)	^3H released (%)	^{32}P released (%)
EDTA (1.0 mM)				
Extract of uninfected *Bacillus subtilis*	35.0	0	0	0
Extract of phage PBS2-infected *B. subtilis*	0	0	0	0
$CaCl_2$ (6.0 mM)				
Extract of uninfected *B. subtilis*	48.4	28.6	31.2	48.0
Extract of phage PBS2-infected *B. subtilis*	0	2.3	33.4	51.2

[a] Double-labeled phage PBS2 DNA (15 nmol) was incubated with 3.8 mg of protein from dialyzed SB5 crude extract in the presence of 1.0 mM EDTA and 10 mM Tris-hydrochloride buffer, pH 8.0, in a total volume of 3.0 ml. Incubation was at 37°C for 45 min, after which the mixture was extracted with buffered, distilled phenol three times and dialyzed against 10 mM Tris-hydrochloride, pH 8.0. Measurement of radioactivity in the acid-soluble fraction of a portion of the reaction mixture before phenol extraction showed that 75% of the input ^3H was released. The depyrimidinated DNA (0.39 nmol) was incubated in a total volume of 0.25 ml with 0.04 mg of protein from uninfected SB5 crude extract or with 0.20 mg of protein from an extract of PBS2-infected SB5 in the presence of either 1.0 mM EDTA or 6.0 mM $CaCl_2$. A control incubation (0.25 ml) contained 0.75 nmol of double-labeled native PBS2 DNA instead of depyrimidinated DNA. The incubations were at 37°C for 45 min. Reactions were terminated and radioactivity as ^3H or ^{32}P was measured in the acid-soluble fractions as described in the text.

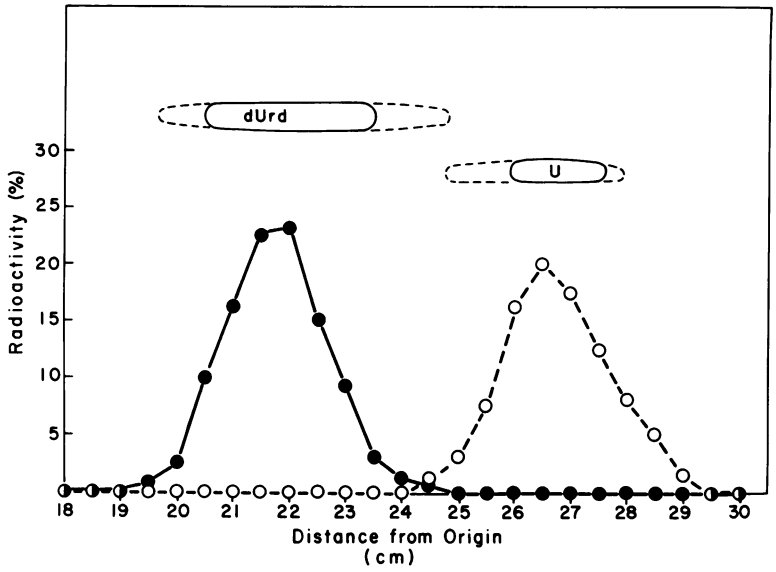

FIG. 3. *Paper chromatographic analysis of the acid-soluble products of PBS2 DNA with B. subtilis extract in the presence of $CaCl_2$. Incubation conditions were as described in the legend to Fig. 2, except that 6.0 mM $CaCl_2$ was present instead of EDTA. During the incubation, 98% of the ^3H and 46% of the ^{32}P were rendered acid soluble. Chromatography was as described in Fig. 2. The sample applied to the paper contained 29,000 cpm of ^3H and 1,250 cpm of ^{32}P. Symbols: (●) [^3H]deoxyuridine marker; (○) acid-soluble ^3H radioactivity.*

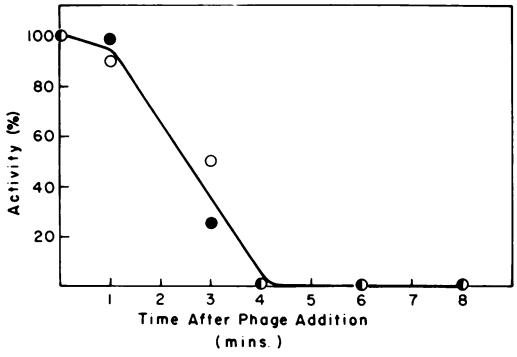

FIG. 4. *Kinetics of inhibition of N-glycosidase and nuclease activities after phage PBS2 infection in vivo. Incubation mixtures (0.20 ml) contained 1.7 nmol of double-labeled PBS2 DNA; 25 mM NaCl; 1 mg of bovine serum albumin per ml; 10 mM Tris-hydrochloride buffer, pH 8.0, and either 1 mM EDTA or 6 mM $CaCl_2$. The extracts of uninfected and phage-infected cells were the same as those used for a previous study (2) and had been stored frozen at −20°C. The same amount of extract (0.05 ml) was used as in the previous study and was shown to be in the linear part of enzyme concentration curves for both N-glycosidase and nuclease activities with the amount of substrate used in the present experiment. One hundred percent of N-glycosidase activity represents 4,820 cpm of ^3H in the acid-soluble fraction, and 100% of nuclease activity represents 7,448 cpm of ^{32}P. Symbols: (○) N-glycosidase activity; (●) nuclease activity.*

DISCUSSION

Published reports on the enzymatic degradation of uracil-containing DNA have in some instances identified nuclease activity and in others *N*-glycosidase activity (2, 6, 8, 10, 11). The object of the present investigation was to determine whether or not both of these activities could be established in unfractionated extracts of *B. subtilis*.

The results confirm previous contentions that in the absence of divalent cation and under conditions that preclude nucleoside phosphorylase activity, the release of free uracil from uracil-containing DNA is attributed to an *N*-glycosidase. Under these conditions of incubation, no nuclease activity is detected, as evidenced by the failure to generate acid-soluble ^{32}P from ^{32}P-labeled PBS2 DNA. The existence of endonuclease activity that specifically attacks uracil sites in the presence of EDTA is not directly excluded. However, our data suggest that if both an *N*-glycosidase and an endonuclease recognize uracil residues in DNA as substrate, most, if not all, activity observed is accounted for the former enzyme.

In the presence of $CaCl_2$, there is significant formation of acid-soluble ^{32}P product from double-labeled PBS2 DNA by extracts of either *B. subtilis* or *E. coli*. These results argue for the presence of a nuclease activity, confirming the

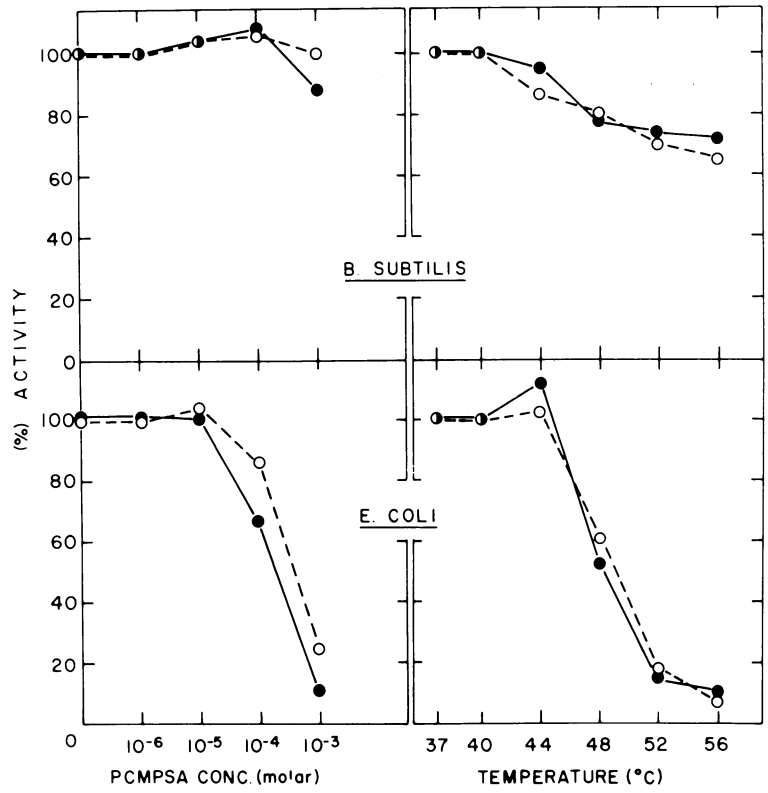

FIG. 5. *Relative inhibition of N-glycosidase and nuclease activities in extracts of B. subtilis and E. coli by heat and by p-chloromercuriphenylsulfonic acid (PCMPSA). Reaction mixtures (0.2 ml) contained 0.86 nmol of double-labeled PBS2 DNA; 1 mM EDTA (for N-glycosidase activity measurement) or 6 mM $CaCl_2$ (for nuclease activity measurement); 25 mM NaCl; 1 mg of bovine serum albumin per ml, 10 mM Tris-hydrochloride buffer, pH 8.0, and 0.35 mg of protein from a dialyzed crude extract of strain SB5 of B. subtilis, or 1.2 mg of protein from a dialyzed crude extract of E. coli W3110. These concentrations of protein were within the linear range of enzyme concentration curves for both N-glycosidase and nuclease activities. Incubations were at 37°C for 30 min. Heat inactivation was carried out by heating 0.2-ml portions of the extracts at the indicated temperatures for 25 min before the incubation at 37°C. Symbols: (○) nuclease activity; (●) N-glycosidase activity.*

contention of W. Carrier and R. B. Setlow (Fed. Proc. 33:1599, 1974) and of Tomita and Takahashi (10, 11). This nuclease activity appears to be specific for PBS2 DNA since only slight degradation of *E. coli* DNA is observed. More significantly, the enzyme that attacks PBS2 DNA is inactive in the presence of extract of phage-infected cells, whereas that which attacks *E. coli* DNA retains its activity. However, the results of our present experiments lead us to the suggestion that the *N*-glycosidase and nuclease activities may function in sequential reactions that result in the loss of free uracil followed by endonucleolytic attack at depyrimidinated sites, in which case the nuclease is not specific for uracil-containing DNA but rather for apyrimidinic DNA. Whether or not this nuclease is the same as the endonuclease that attacks apurinic sites in DNA (12, 13) remains to be determined. The *N*-glycosidase is currently about 600-fold purified and can be readily separated from any associated nuclease activity (J. Duncan, L. Hamilton, and E. C. Friedberg, unpublished observations).

This postulated series of enzymatic events is analogous to that observed in the degradation of alkylated DNA in vitro. Friedberg and Goldthwait (3) described an activity in extracts of *E. coli* which attacks DNA alkylated with methylmethanesulfonate. Subsequent studies have resolved endonuclease II from an endonuclease active on depurinated DNA (5). Endonuclease II possesses an *N*-glycosidic activity as evidenced by its ability to release 3-methyladenine from DNA treated with dimethylsulfate and O^6-methylguanine, 3-methyladenine, 1-methyladenine, and 7-methyladenine from DNA treated with methylnitrosourea (5). In addition, endo-

nuclease II possesses endonucleolytic activity on alkylated DNA. At present it is not clear whether the two activities are functions of a single enzyme; however, Kirtikar et al. (5) have offered a number of lines of evidence suggesting the unlikelihood that the endonucleolytic component of endonuclease II represents contamination by a distinct apurinic endonuclease. On the other hand, Lindahl (8) has presented evidence suggesting that the endonucleolytic component of what he characterized as endonuclease II can be distinguished from an *N*-glycosidic activity that removes 3-methyladenine from alkylated DNA. Both of these activities are clearly separable from the *E. coli N*-glycosidase that attacks uracil residues in DNA (6).

It is tempting to speculate that both the enzymatic system that attacks uracil-containing DNA and that degrading alkylated DNA are representative of an alternative mode of excision repair of damaged or altered DNA. Accordingly, damaged or inappropriate bases could be removed from polynucleotide chains by direct endonucleolytic attack or by enzyme-catalyzed base loss followed by endonucleolytic attack at the site of the lost base. Since both of these repair modes are examples of excision repair, we propose that the repair mode involving excision of damaged nucleotides be designated as nucleotide excision repair and that involving excision of damaged bases as base excision repair.

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