Mutagenic Nucleoside Analog N^4-Aminocytidine: Metabolism, 
Incorporation into DNA, and Mutagenesis in *Escherichia coli*

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N^4-Aminocytidine, a nucleoside analog, is strongly mutagenic to various organisms including *Escherichia coli*. Using *E. coli* WP2 (trp), we measured the incorporation of [5-^3H]N^4-aminocytidine into DNA and at the same time measured the frequency of reversion of the wild type, thereby attempting to correlate the incorporation with mutation induction. First, we observed that N^4-aminocytidine uptake by the *E. coli* cells was as efficient as cytidine uptake. High-pressure liquid chromatographic analysis of nucleoside mixtures obtained by enzymatic digestion of isolated cellular DNA showed that the DNA contained [^3H]N^4-aminodeoxyctydine, corresponding to 0.01 to 0.07% of the total nucleoside; the content was dependent on the dose of N^4-aminocytidine. There was a linear relationship between the N^4-aminocytidine content in DNA and the mutation frequency observed. These results constitute strong evidence for the view that the N^4-aminocytidine-induced mutation in *E. coli* is caused by the incorporation of this agent into DNA as N^4-aminodeoxyctydine.

We also found that the major portion of radioactive DNA in cells that had been treated with [5-^3H]N^4-aminocytidine was in the deoxyctydine fraction. We propose a metabolic pathway for N^4-aminocytidine in cells of *E. coli*. This pathway involves the formation of both N^4-aminodeoxyctydine 5'-triphosphate and deoxyctydine 5'-triphosphate; the deoxyctydine 5'-triphosphate formation is initiated by conversion of N^4-aminocytidine into uridine. In support of this proposed scheme, a cytidine deaminase preparation obtained from *E. coli* catalyzed the decomposition of N^4-aminocytidine into uridine and hydrazine.

**MATERIALS AND METHODS**

**Bacterial strains and media.** The following strains were provided by B. J. Bachmann (*E. coli* Genetic Stock Center, Yale University, New Haven, Conn.): *E. coli* Shl77 (leuB6 hisG1 dcd-1 cdd-1 argG6 metB1 lacY1 gal-6 malA1 xyl-7 mlt-2 rpsL104 tonA2 tss-1 λ′ supE44), which was constructed by J. Neuhard; JC411 (parent of Shl77; cdd'/dcd') (1); Sh441 (cdd-5 upp-11 relA1 metB1 rpsL254); and Sh422 (parent of Sh441; cdd− his udk) (6). GM33 (dam−) and its parent, GM28 (19), were a gift of C. Janion of the Institute of Biochemistry and Biophysics, Poland. *E. coli* WP2 (trp), WP2 (trp, uvrA), and HF4704 (thy) were also used (9).

L broth (1 liter) contained 10 g of Polypeptone (Daigo-Eiyo, Osaka, Japan), 10 g of Bacto-Tryptone (Difco Laboratories, Detroit, Mich.), 5 g of yeast extract, 1 g of glucose, and 5 g of NaCl. NB medium (1 liter) contained 8 g of nutrient broth (Difco) and 5 g of NaCl.

**Materials.** [^3H]N^4-aminocytidine was prepared as described previously (9, 10) from [5-^3H]cytidine (ICN Pharmaceuticals Inc., Irvine, Calif.); the specification of this material states that it might contain up to 5% [6-^3H]cytidine. Ethyl acetamidate hydrochloride was a product of Eastman Kodak Co. (Rochester, N.Y.). Derivatization of N^4-aminocytidine compounds by this reagent was carried out as described previously (10). DNase I and RNase A were obtained from Sigma Chemical Co. (St. Louis, Mo.), RNase T1 was from Sankyo (Japan), and nuclease P1 was from Yamasa (Japan). Venom phosphodiesterase and *E. coli* alkaline phosphatase were obtained from Cooper Biomedical, Inc. (West Chester, Pa.). 2-Oxopirimidine riboside was a gift of M. Maeda of the National Cancer Center Research Institute, Japan.

**N^4-Aminocytidine uptake and effect on cell growth.** *E. coli* HF4704 (thy) was grown for 2 h at 37°C in a ∝XC medium (9) supplemented with 2 μg of thymine per ml and [^3H]N^4-aminocytidine (20 Ci/mol). In the control culture, in which no N^4-aminocytidine was present, the cell number was 4.3 × 10^9/ml at the start and 9.1 × 10^9/ml at the end of the incubation. The cells were counted, collected, washed twice with 10 mM Tris hydrochloride–0.8% NaCl (pH 8), and lysed for radioactivity measurements. For comparison, cytidine uptake and its effect on cell growth were determined in a similar manner.

**Assays for cytidine deaminase.** Cytidine deaminase activity was detected in a sample of *E. coli* extract by a modification of the procedure of Cohen and Wollenden (2). Briefly, after

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the streptomycin precipitation and heat treatment of an extract from E. coli WP2 (trp), the proteins were precipitated by the addition of ammonium sulfate (72% saturation), collected, and fractionated by DE52 (Whatman) column chromatography. The peak fraction of cytidine deaminase activity was used for studies of N^4-aminocytidine degradation. The degradation kinetics were investigated spectrophotometrically (2). The reaction mixtures contained various concentrations of N^4-aminocytidine and 0.03 U of cytidine deaminase per ml in 50 mM Tris hydrochloride (pH 7.6), and the changes in A_{282} were monitored. High-pressure liquid chromatographic (HPLC) analysis of the decomposition products was done with an Inertsil 5-μm (4.6 by 150 mm) ODS column (Gasukuro Kogyo Inc., Tokyo, Japan) with 50 mM formic acid–2% methanol as the eluate at a flow rate of 0.5 ml/min.

**Incorporation of N^4-aminocytidine into cellular DNA.** A 0.5-ml sample of an overnight culture of E. coli WP2 (trp uvrA) was added to 10 ml of L broth, and the bacteria were grown for one generation as judged by the A_{660} value. [3H]N^4-aminocytidine was then added at a concentration of 10 μM (or 1 or 5 μM) (50 to 60 Ci/mol), and the mixture was incubated for 20 min at 37°C. In the time course studies, NB medium was used in place of the L broth. After incubation, the bacteria were collected by centrifugation and washed twice with phosphate-buffered saline, pH 7.2 (PBS). The pellet was suspended in PBS, and a portion was taken for the measurement of mutation frequency. The cell suspension was pelleted, and the cells were suspended in 10 mM Tris hydrochloride–0.8% NaCl–10 mM EDTA–0.15 mg of lysozyme per ml (pH 7) and allowed to stand at room temperature for 10 min. After the addition of sodium lauryl sulfate to a final concentration of 1%, the mixture was incubated for a few minutes to achieve complete lysis of the cells. DNA was prepared by extraction with phenol followed by chloroform. The DNA was recovered by ethanol precipitation and further subjected to digestion with a mixture of RNase A and RNase T_1, followed by phenol extraction and ethanol precipitation. The DNA was dissolved in 10 mM Tris hydrochloride–1 mM EDTA (pH 7.5), and the phenol was removed by extensive washing of the solution with ether.

For the identification of incorporated radioactive deoxyribonucleotides, the DNA (1 A_{260} unit) was digested with a mixture of 25 μg of pancreatic DNAse I, 0.8 U of snake venom phosphodiesterase, and 0.3 U E. coli alkaline phosphatase in 0.2 M Tris hydrochloride–4 mM MgCl_2 at pH 8 for 2 h at 37°C. The resulting mixture of deoxyribonucleosides was derivatized with ethyl acacetimide (a reagent specific for N^4-aminocytosine), giving rise to the formation of a triazolopyrimidine derivative (10). Both before and after the derivatization, the samples were analyzed by HPLC on an Inertsil ODS column (identical as the one used for the cytidine deaminase assay). For elution, a linear gradient was used: 50 mM formic acid at 0 min and 70% 50 mM formic acid–30% methanol at 60 min. The flow rate was 0.5 ml/min, and fractions were collected every 30 s for radioactivity measurements.

**Measurement of mutation frequency.** For the reversion assays with strains of E. coli WP2, 0.2 ml of treated bacterial cells suspended in PBS was plated with 3 ml of top agar onto a minimal medium–glucose plate supplemented with 160 μg of nutrient broth (5) per ml. The cell suspension was also diluted 4 × 10^3-fold with PBS, and 0.1 ml of the diluted sample was plated with 3 ml of top agar supplemented with 0.33 mg of tryptophan per ml for scoring the surviving cells. Mutation assays with drug resistance as markers were done as described previously (19).

**Requirement of cell proliferation for mutation induction and for N^4-aminocytosine incorporation into DNA.** To 10 ml of L broth was added 0.5 ml of an overnight culture of E. coli WP2 (trp uvrA); the mixture was incubated at 37°C for 40 min, during which period the A_{660} increased twofold. The cells were collected, washed twice with PBS, and suspended in 10 ml of PBS or L broth. The A_{660} was recorded, and the suspension was incubated at 37°C for 35 min. [3H]N^4-aminocytidine was added at a final concentration of 10 μM (127 Ci/mol), and the mixture was further incubated for 25 min. The A_{660} was recorded, and the cells were collected, washed, and subjected to the measurement of mutation frequency and DNA analysis.

**RESULTS**

**Uptake, metabolism, and catabolism of N^4-aminocytidine in E. coli.** We supposed that this mutagenic nucleoside is transported, utilized, metabolized, and catabolized in E. coli in a way similar to cytidine (Fig. 1). The uptake of N^4-aminocytidine into the bacterial cells occurred as efficiently as that of cytidine (Fig. 2). This similarity suggests that N^4-aminocytidine is transported and utilized like a normal nucleoside. N^4-Aminocytidine was only slightly more growth inhibiting than cytidine (Fig. 2).

The enzyme that processes N^4-aminocytidine may be either cytidine deaminase or uridine-cytidine kinase. E. coli
strains lacking one or both of these enzymes were grown in media containing [5-3H]N4-aminocytidine, and the DNA was extracted. The radioactivity in DNA from Sdot177, a cytidine deaminase-lacking strain, was 14% of the activity of DNA from the wild-type strain JC411. The radioactivity of DNA from another cytidine deaminase-lacking strain (Sdot441) was similarly smaller (13%) than that of JC411. In the uridine-cytidine kinase-deficient mutant Sdot422, essentially no radioactivity was detected in DNA. As described below (Fig. 3), most of the radioactivity in the DNA of the wild-type strain was found in the deoxycytidine fraction. These results suggest that the major metabolic and catabolic pathway of N4-aminocytidine is decomposition to uridine, followed by phosphorylation of the uridine with uridine-cytidine kinase, leading to the formation of UTP, CTP, dCTP, and TTP, and subsequent incorporation of these triphosphates into nucleic acids (Fig. 1).

Decomposition of N4-aminocytidine by cytidine deaminase. As described above, the incorporation of N4-aminocytidine into DNA of the strains lacking cytidine deaminase was poor. This finding suggests that in E. coli cells cytidine deaminase can catabolize N4-aminocytidine to uridine. Therefore, we studied the catalytic action of E. coli cytidine deaminase on N4-aminocytidine and compared the kinetic data obtained with those for cytidine.

N4-Aminocytidine was indeed decomposed by this enzyme preparation to uridine and hydratide in vitro. When the reaction mixture was subjected to HPLC analysis, uridine was found to be the sole product with A250. Neither cytidine nor 2-oxopyrimidine riboside, possible products of N4-aminocytidine degradation, was detected even in an extensively degraded sample of N4-aminocytidine (data not shown). The hydrazide formation was demonstrated by determining the titers of the reaction mixture with p-dimethylbenzaldehyde (23). The amount of hydrazide formed corresponded to the amount of uridine produced.

Under the conditions described in Materials and Methods, the apparent \( K_m \) for N4-aminocytidine was 1.39 mM, whereas the observed \( K_m \) for cytidine was 0.20 mM, a value similar to that reported in the literature (2). In contrast, the difference in the \( V_{max} \)s was small: the ratio of the \( V_{max} \)s for the decompositions of N4-aminocytidine and cytidine was 1: 2. 2-Oxopyrimidine riboside, a known inhibitor of E. coli cytidine deaminase (8), inhibited the decomposition of N4-aminocytidine as well as that of cytidine.

Cytidine deaminase from E. coli also has been shown to catalyze the reverse reaction in vitro, i.e., the formation of cytidine or its derivatives from uridine and ammonia or other amines (3). Therefore, it may be expected that the deaminase can catalyze the formation of N4-aminocytidine from uridine and hydratide. A reaction mixture containing 10 mM uridine, 2 M hydratide, and 22 U of cytidine deaminase per ml at pH 9.2 was incubated for 50 h at 22°C. Analysis of the reaction mixture with HPLC showed that 0.4 mM N4-aminocytidine was formed. The amount of N4-aminocytidine in the reaction mixture lacking either hydratide or the deaminase was less than the limit of detection (<0.01 mM).

Correlation of N4-aminodeoxycytidine content in DNA with mutation frequency. DNA was extracted from E. coli WP2 (trp uvrA) cells grown in the presence of [3H]N4-aminocytidine and digested to deoxyribonucleosides. After derivatization with ethyl acetamidate, the digest was fractionated by HPLC. A typical HPLC profile is shown in Fig. 3. The triazolopyrimidine derivative of N4-aminodeoxycytidine was detected as a well-separated peak. From the radioactivity of the peak and the amounts of four major deoxyribonucleosides, the N4-aminodeoxycytidine content was calculated to be 0.01% of the total deoxyribonucleosides. The sample before the ethyl acetimidate treatment was also analyzed by HPLC. As expected, no radioactivity was found in the fractions at the retention time for the triazolopyrimidine derivative. Some radioactivity was present in the peak for thymidine (Fig. 3). This may be due to the presence of [6-3H]thymidine, which can be formed from [6-3H]cytidine that contaminated the sample of [5-3H]cytidine. Other unidentified peaks might be those of ribonucleosides, ribonucleotides, and deoxyribonucleotides. The incorporation of N4-aminocytidine as N4-aminocytosine into DNA was further confirmed by analyzing a nucleotide mixture prepared by digestion with nuclease P1 of DNA from E. coli cultured in the presence of [3H]N4-aminocytidine. Thus, the nucleotide mixture was derivatized with ethyl acetamidate and subjected to HPLC fractionation on an ion-exchange column (Partisil 10SAX; Whatman, Inc., Clifton, N.J.). A radioactive fraction corresponding to the triazolopyrimidine nucleotide was obtained, and the amount of the radioactivity found was consistent with the results found in the analysis of the nucleoside mixture (data not shown).

Figure 4 shows the mutation frequency and the N4-aminodeoxycytidine content of DNA found in E. coli WP2 (trp uvrA) cultured for various lengths of time with 10 \( \mu \)M [3H]N4-aminocytidine. At each desired time, a portion of the culture was taken and plated to score Trp* revertants, and another portion was used to extract DNA and measure its N4-aminodeoxycytidine content. Both the mutation frequency and the N4-aminodeoxycytidine content reached a maximum at 20 min and then decreased gradually. This decrease may be due to the depletion of N4-aminocytidine in the medium as a result of consumption by the growing bacteria.

E. coli WP2 (trp uvrA) was cultured in media containing N4-aminocytidine at different concentrations for 20 min, and the mutation frequency and the N4-aminodeoxycytidine content in DNA were measured. Both the mutation frequency and the N4-aminodeoxycytidine content increased as a function of the N4-aminocytidine concentration (Fig. 5).
To confirm that N4-aminocytidine is an incorporation-type mutagen, an experiment was done in which cells of E. coli WP2 (trp uvrA) were treated with radioactive N4-aminocytidine either in PBS, in which the cells would proliferate only poorly, or in a normal growth medium. The treatment in PBS gave rise to mutagenesis at a much lower level compared with that obtained in the growth medium (Table 1). Consistent with the low mutation frequency, the content of N4-aminodeoxycytidine in DNA was also low.

**DNA repair and N4-aminocytidine-induced mutagenesis.** As reported previously (9), the number of Trp+ revertants per plate produced from E. coli WP2 (trp uvrA), a UvrABC excision-repair-deficient strain, on treatment with N4-aminocytidine is three times greater than that produced from E. coli WP2 (trp uvrA).

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**FIG. 3.** Analysis of [3H]N4-aminodeoxycytidine in DNA digest from E. coli WP2 (trp uvrA) grown in the presence of [3H]N4-aminocytidine: fractionation of deoxyribonucleosides by HPLC after the modification with ethyl acetimidate. Nonradioactive samples of N4-aminocytidine (CamR), N4-aminodeoxycytidine (dCamR), 5-oxo-5,6-dihydro-s-triazolo[4,3-c]pyrimidine riboside (imCamR), and 5-oxo-5,6-dihydro-s-triazolo[4,3-c]pyrimidine deoxyriboside (imdCamR) were added as markers before injection. The arrow indicates the radioactivity peak for 5-oxo-5,6-dihydro-s-triazolo[4,3-c]pyrimidine deoxyriboside.

**FIG. 4.** Relationship between mutation frequency, N4-aminodeoxycytidine content in DNA, and cell proliferation in cultures of E. coli WP2 (trp uvrA) treated with [3H]N4-aminocytidine. The mutation frequency measured before the addition of N4-aminocytidine was $6 \times 10^{-8}$.

**FIG. 5.** Mutation frequency and N4-aminodeoxycytidine content of DNA in E. coli WP2 (trp uvrA) grown at various concentrations of N4-aminocytidine for 20 min. The mutation frequency in the absence of N4-aminocytidine was $2 \times 10^{-8}$.
TABLE 1. Requirement of cell proliferation for incorporation of $N^4$-aminodeoxycytidine into DNA and induced mutagenesis$^a$

<table>
<thead>
<tr>
<th>Medium</th>
<th>$A_{600}$ at:</th>
<th>$N^4$-Amino-deoxycytidine in DNA$^b$</th>
<th>Mutation frequency$^c$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>60 min</td>
<td></td>
</tr>
<tr>
<td>PBS</td>
<td>0.42</td>
<td>0.39</td>
<td>1.1</td>
</tr>
<tr>
<td>L broth</td>
<td>0.37</td>
<td>0.62</td>
<td>3.9</td>
</tr>
</tbody>
</table>

$^a$ See Materials and Methods for experimental details.
$^b$ Residues in 10$^4$ nucleotides.
$^c$ Frequency of mutation from Trp$^-$ to Trp$^+$ per 10$^4$ cells. The frequency before the treatment was 0.0004.

coli WP2 (trp), an excision-proficient control. These two strains were cultured in 10 $\mu$M $N^4$-aminocytidine for 1 h, and the $N^4$-aminodeoxycytidine contents in DNA were measured. The content in the excision-repair-deficient strain was 50% greater than that in the wild-type strain. This result suggests that some of the $N^4$-aminocytidine in DNA is excised via the UvrABC system.

The mismatch repair system has been regarded as the most effective repair mechanism against mutations induced by base analogs (5, 16, 17, 19). It has been reported that this repair can efficiently act to reduce the mutational rates in the mutagenesis induced by either 5-bromouracil, 2-aminopurine (5, 16, 17), or $N^4$-hydroxy-cytidine (19). The mutagenicity and cytotoxicity of $N^4$-aminocytidine in GM33 (dam-3) were measured and compared with those in a parental strain, GM28. Both the mutation frequency and the cytotoxicity in the dam strain were greater than those in the wild-type strain (Table 2). However, the mutation frequency differences were much smaller than those observed in the mutation by $N^4$-hydroxy-cytidine. The mutL mutation also enhanced the mutagenic response to $N^4$-aminocytidine, but again the effect was not great (data not shown).

DISCUSSION

We have studied here in a quantitative manner the relationship between $N^4$-aminodeoxycytidine content in DNA and mutation frequency. Figure 6 is a replotting of the data shown in Fig. 4 and 5. The mutation frequency increased linearly as a function of $N^4$-aminodeoxycytidine content in DNA. This fact, together with the dependence of $N^4$-aminocytidine incorporation into DNA on cell proliferation (Table 1), demonstrates that $N^4$-aminocytidine-induced mutations are caused by errors occurring during the incorporation of $N^4$-aminodeoxycytidine triphosphate into DNA and/or during the replication of DNA with $N^4$-aminocytidine residues.

Incorporation of mutagenic nucleoside analogs into DNA of bacteria (15, 16, 18) and of mammalian cells (7, 13) has been the subject of intensive studies in several laboratories. The incorporation of 2-aminopurine and $N^4$-hydroxy-cytidine into E. coli DNA was shown to be too small for their contents to be estimated (14, 22). Although a direct comparison is difficult, the results of our study suggest that $N^4$-aminocytidine can be incorporated into DNA more readily than 2-aminopurine and $N^4$-hydroxy-cytidine. This would explain why this amino-derivative of cytidine is more strongly mutagenic than the hydroxyl derivative (9), in spite of the fact that the corresponding triphosphates, i.e., $N^4$-aminodeoxycytidine 5'-triphosphate and $N^4$-hydroxy-deoxycytidine 5'-triphosphate, have similar mutagenic potency (20).

5-Bromouracil and 5-bromodeoxyuridine are incorporated into DNA very efficiently. However, only low mutation frequencies were obtainable with these reagents (4, 7, 16, 17). The low activities might be due to a low error frequency per single incorporation event, because the minor enol form of 5-bromouracil is supposed to be very rare. More importantly, the mismatch repair system may remove specifically those particular bromouracil residues that will cause mutations (16, 17). In 2-aminopurine and $N^4$-hydroxy-cytidine mutagenesis, mutation frequencies are also shown to be much greater in mismatch-repair-deficient strains, and these agents are more toxic in these strains (5, 19).

In contrast, in the case of $N^4$-aminocytidine, no great

TABLE 2. $N^4$-Aminocytidine-induced mutation in mismatch-repair-deficient E. coli

<table>
<thead>
<tr>
<th>Mutagen</th>
<th>Dose (\muM)</th>
<th>Mutation frequency$^a$</th>
<th>Frequency ratio (dam/dam$^+$)</th>
<th>Viable cells (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GM33 (dam)</td>
<td>GM28 (dam$^+$)</td>
<td>GM33 (dam)</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
<td>1.0</td>
<td>0.1</td>
<td>10</td>
</tr>
<tr>
<td>$N^4$-Aminocytidine$^b$</td>
<td>0.2</td>
<td>2.3</td>
<td>0.7</td>
<td>3.3</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>6.6</td>
<td>1.8</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>38</td>
<td>9.2</td>
<td>4.1</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>98</td>
<td>41</td>
<td>2.4</td>
</tr>
<tr>
<td>$N^4$-Hydroxy-cytidine$^b$</td>
<td>200</td>
<td>9.1</td>
<td>0.2</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>14.1</td>
<td>0.5</td>
<td>28</td>
</tr>
</tbody>
</table>

$^a$ Frequency of mutation from Rif$^-$ to Rif$^+$ per 10$^6$ viable cells.
$^b$ Incubation was 4 h in NB medium at 37°C.

FIG. 6. Correlation of $N^4$-aminodeoxycytidine content in DNA with mutation frequency.
differences were observed with respect to the mutation frequencies and the growth inhibitions between the mismatch-repair-deficient and proficient strains (Table 2). Therefore, it appears that N⁴-aminocytosines in DNA can be removed by a mismatch repair system, but the removal is less efficient than for the other base analogs. This could be a reason why N⁴-aminocytidine is a strong mutagen.

Our results suggest that the UvrABC system can excise the lesions caused by N⁴-aminocytidine. It is known that other base analogs (5-bromouracil, 2-aminopurine, and N⁴-hydroxycytidine) are not affected by UvrABC. It is difficult to explain why N⁴-aminocytosine is different from other analogs in this regard, because N⁴-aminocytosine, like these other analogs, has a structure close to a normal base, in this case to cytosine. The possibility that N⁴-aminocytosines in DNA make reversible adducts with molecules in the cell is not excluded, since the hydrazino group can react with ketonic groups. The adducts thus formed might be recognized by UvrABC. A more favorable explanation is that N⁴-aminocytidine might induce UvrABC very efficiently, and the induced enzymes might excise this analog.

We showed that E. coli cytidine deaminase can produce N⁴-aminocytidine from uridine and hydrazine. Hydrazine is a weak mutagen for E. coli (24), in contrast to the strong mutagenicity of N⁴-aminocytidine. An interesting possibility is that a part of the mutagenicity of hydrazine in E. coli may be due to N⁴-aminocytidine formed from hydrazine and uridine by this enzymatic reaction.

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LITERATURE CITED