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A DEOXYTHYMIDINE KINASE-DEFICIENT MUTANT OF *ESCHERICHIA COLI*

I. ISOLATION AND SOME PROPERTIES

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SUMMARY

A new mutant (*tdk–1*) of *Escherichia coli* strain K12 incapable of incorporating [2-^{14}C]deoxythymidine into its DNA, while exhibiting normal growth in the medium without deoxythymidine, was isolated. It was found that the mutant lacks the activity of deoxythymidine kinase (ATP:thymidine 5′-phosphotransferase, EC 2.7.1.21) which catalyzes the conversion of deoxythymidine to deoxythymidine 5′-monophosphate. The mutant was also shown to be unable to incorporate 5-bromodeoxyuridine, and its growth was inhibited by 5-fluorouracil or 5-fluorodeoxyuridine even in the presence of uridine and deoxythymidine in the medium. When mutant cells were infected with T2 or T4 phage, a rapid incorporation of [2-^{14}C]deoxythymidine accompanied by an appearance of deoxythymidine kinase activity was observed. Evidence suggests that the enzyme induced by infection with T4 phage is qualitatively different from the bacterial deoxythymidine kinase and that T4 phage, and presumably other T even phages as well, carries its own structural gene for this enzyme.

INTRODUCTION

Deoxythymidine (dT) kinase (EC 2.7.1.21) is an enzyme that catalyzes the conversion of dT to deoxythymidine 5′-monophosphate (dTMP). The enzyme in *Escherichia coli* has been partially purified and some of its properties have been

Abbreviations: dT, deoxythymidine; dTMP, deoxythymidine 5′-monophosphate; dTDP, deoxythymidine 5′-diphosphate; dTTP, deoxythymidine 5′-triphosphate; dU, deoxyuridine; dBU, 5-bromodeoxyuridine; dFU, 5-fluorodeoxyuridine; FU, 5-fluorouracil; dFUMP, 5-fluorodeoxyuridine 5′-monophosphate; FUMP, 5-fluorouridine 5′-monophosphate; dA, deoxyadenosine. The following gene symbols were used: *tdk*, deoxythymidine kinase; *ts*, temperature sensitivity; *trpE*, anthranilate synthetase; *ton*, resistance to phage T1; *ilv*, requirement for isoleucine and valine; *cysB*, requirement for cysteine; att_{80}, attachment site of phage φ80.

reported[1,2]. More recently, the molecular mechanisms of allosteric transition with this enzyme have been the subject of extensive biochemical study[3,4]. However, the gene(s) controlling the synthesis of this enzyme has not been identified, nor has it been established whether the synthesis of the enzyme is induced by infection with various bacteriophages.

This paper will describe the isolation and characterization of a mutant lacking the dT kinase activity. Abstracts of this work have appeared elsewhere[5,6].

MATERIALS AND METHODS

Bacterial and phage strain

Wild-type strain W3110 of *E. coli* K12 was used as the parental strain for isolation of the mutant. Mutant strains used are as follows; KY608 F$^-$ *tdk-1 ts trpE ton*, KY688 F$^-$ *tdk-1 trp E ilv ton*, KY895 F$^-$ *tdk-1 ilv*. Standard T1, T2, T4D, and T5 phages were also employed.

Chemicals

[2-^{14}C]dT and [^{3}H]deoxyuridine (dU) were purchased from New England Nuclear Corp. and Schwarz BioResearch, Inc. respectively. 5-Bromodeoxyuridine (dBU), dT and ATP were purchased from Sigma Chemical Corp. 5-Fluorodeoxyuridine (dFU) was donated by F. Hoffmann-La Roche & Co., Basle, Switzerland.

Media

A glucose–salts minimal medium used in this study has been described by VOGEL AND BONNER[7]. A peptone–glucose medium contained (per l): polypeptone (Wako Drug Co.) 20 g, NaCl 5 g, glucose 2 g. 1 M NaOH was added to adjust the pH to 7.2.

Incorporation of [^{14}C]dT into DNA

Cells were grown to exponential phase in a glucose–salts minimal medium supplemented with 0.2 % Difco casamino acids, 20 μg/ml L-tryptophan, and 250 μg/ml deoxyadenosine (dA). To this was added 8 μg/ml [2-^{14}C]dT (specific activity, 300 μC/mmole) and the mixture incubated at 30° or 37° with shaking. 1-ml aliquots were taken at various times and added to 3 ml of 3.5 % cold $HClO_4$. After standing 30 min in the cold, cells were centrifuged, washed twice with cold $HClO_4$ and suspended in 5 % aqueous ammonia solution. The whole content was placed on a planchet, dried and the radioactivity counted by a Nuclear-Chicago gas-flow counter.

Enzyme preparation

Bacteria were grown in a glucose–salts medium supplemented with 0.2 % casamino acids and 20 μg/ml of L-tryptophan. Cells were harvested at the late exponential phase, collected by centrifugation, washed and resuspended with 0.05 M

Tris–HCl buffer (pH 7.8) containing 200 μg/ml bovine serum albumin, and sonicated in a Raytheon 10 KC sonic oscillator. After centrifugation for 30 min at 10 000 $\times g$, the supernatant was collected (crude extract). The crude extract (protein, 1–2 mg/ml) was heated in a water-bath at 70° for 5 min with occasional stirring, chilled in ice-water, centrifuged for 15 min at 1000 $\times g$, and the supernatant collected (heated extract). For preparing enzyme extracts from phage-infected bacteria, cells grown in a peptone–glucose medium were infected with phage T4D at a multiplicity of 5. Crude extracts obtained were passed through a column of Sephadex G-25 and the high-molecular-weight fractions collected were used for enzyme assay. The amount of protein contained in the extracts was measured by the method of LOWRY *et al.*[8].

Assay for dT phosphorylase (thymidine : orthophosphate deoxyribosyltransferase, EC 2.4.2.4)

The assay for dT phosphorylase was done by the procedure of KRENITSKY, BARCLAY AND JACQUEZ[9] with minor modifications. The reaction mixture containing 10 mM dT, 0.1 M sodium phosphate buffer (pH 7.4) and crude extract was incubated at 20°. At various times, 0.2-ml aliquots were removed, added to 0.2 ml of 1 M NaOH and diluted to 2 ml with water. The increase in absorbance at 300 mμ was measured by the use of a Shimazu spectrophotometer.

Assay for dT kinase

Activity of dT kinase was measured by the conversion of [2-^{14}C]dT to [^{14}C]dT nucleotides that can be separated from the substrate by column chromatography. The incubation mixture (0.5 ml) containing 35 μmoles Tris–HCl buffer (pH 7.8), 1.4 μmoles $MgCl_2$, 0.35 μmole $MnCl_2$, 2.8 μmoles ATP, 0.42 μmole [2-^{14}C]dT (360 μC/mmole, purified through a DEAE-cellulose column), 140 μg bovine serum albumin (Fraction V, Nutritional Biochemical Corp.), and enzyme (0.2 ml of crude or heated extract) was incubated at 37° unless otherwise indicated. Assay of the product was carried out according to the following procedure (Y. SUGINO, personal communication). At various times during incubation, assay tubes were taken out, 1 ml distilled water added and the tubes heated for 5 min in a boiling-water bath to stop the reaction, chilled in ice-water, and centrifuged at 1000 $\times g$ for 15 min. An aliquot from the supernatant was passed through a small column (5 mm $\times$ 20 mm) of DEAE-cellulose, previously equilibrated with 0.01 M Tris–HCl (pH 7.5). The column was washed with 5 ml of distilled water and the [^{14}C]nucleotides were eluted with 2 ml of 0.2 M NaCl. The eluate was dried on a planchet and the radioactivity counted by use of a gas-flow counter.

Analysis of reaction products by paper chromatography

The reaction mixture was boiled 5 min to stop the reaction, centrifuged, and the supernatant passed through a column of acidified charcoal in order to remove salts. After washing with distilled water, the material absorbed on to the charcoal was eluted with alcoholic ammonia (50 % ethanol containing 0.084 % ammonia). The eluate was concentrated by use of a rotary evaporator, and spotted on to a filter

paper (Toyo roshi 51A, 40 cm × 40 cm) with markers of authentic dT, thymine, dTMP, deoxythymidine 5′-diphosphate (dTDP) and deoxythymidine 5′-triphosphate (dTTP). The descending chromatography was developed by a solvent containing isobutyric acid, 1 M ammonium hydroxide, and 0.1 M EDTA (100:60:1.6, v/v/v)[10] at room temperature. The paper was dried, checked for ultraviolet-absorbing spots, and cut off into 5-mm pieces. The radioactivity of each piece was measured with the aid of a gas-flow counter. In this chromatography, dT, thymine, dTMP, dTDP, and dTTP were separated from one another except that thymine overlapped with dT. The fraction containing dT and thymine was eluted overnight in distilled water, and the eluate was used for the second chromatography which was developed in distilled water[11]. dT and thymine could be separated from each other by this procedure.

Irradiation with near-ultraviolet light of cells that had incorporated 5-bromodeoxyuridine (dBU)

An overnight culture in a glucose–salts minimal enriched with 0.2 % casamino acids and 50 μg/ml L-tryptophan was diluted 20-fold in the same medium, supplemented with 250 μg/ml dA and 50 μg/ml dBU (or dT as a control), and aerated at 37° for 4 h. Cells were harvested by centrifugation, washed and resuspended in saline solution and were irradiated in a petri dish with a fluorescent lamp (National, FL20E, maximal intensity at 305 mμ) at a distance of 10 cm. Samples were taken at several intervals, diluted and plated onto a peptone–glucose agar for the determination of cells which had survived.

RESULTS

Isolation of the mutant

In the course of examining temperature-sensitive mutants of *E. coli* strain W3110, we came across a mutant totally incapable of incorporating [^{14}C]dT into the acid-insoluble fraction at either high or low temperature. This mutant, designated as strain KY608, was originally isolated as a mutant that can grow at 30° but not at 42° on peptone–glucose agar from the population of cells treated with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine followed by penicillin screening at 42°. Besides its temperature sensitivity, this mutant requires tryptophan for growth, is resistant to T1 phage and, as shown below, is unable to incorporate [^{14}C]dT into the acid-insoluble form. Since it was found that the tryptophan requirement is satisfied either by indole or anthranilic acid, it cannot be a deletion mutant of the classical type, *i.e.* a T1-resistant tryptophan auxotroph[12]. It thus appears that strain KY608 differs from the parental strain with respect to at least 4 characters due to 4 independent mutations induced by a single treatment with the mutagen.

Incorporation of [^{14}C]dT in vivo

It can be seen in Fig. 1 that the mutant, in contrast to the parental wild-type strain, failed to incorporate appreciable amounts of [^{14}C]dT into the acid-insoluble

form, while its growth was essentially normal at 30°. The possibility that [^{14}C]dT was converted by the mutant to a form that could not be taken up by the cells, was made unlikely by the observations that the radioactivity remaining in the mutant culture filtrate could still be adsorbed onto charcoal and rapidly taken up by wild-type cells.

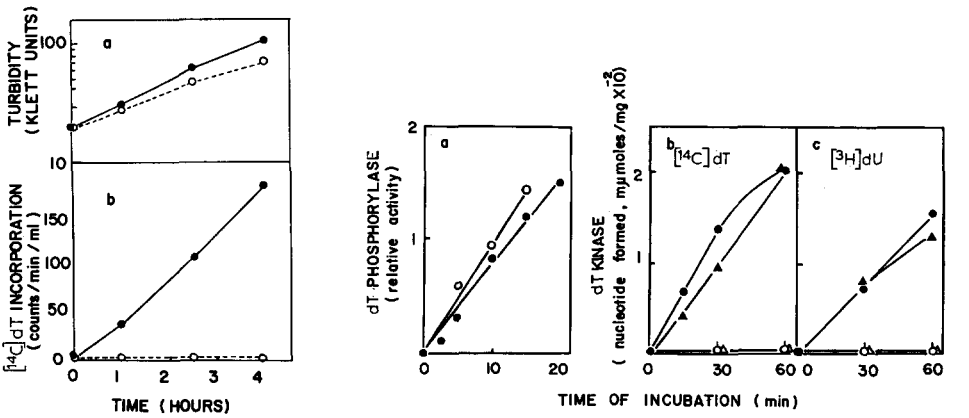

Fig. 1. Incorporation of [^{14}C]dT in wild-type (W3110) and mutant strain KY608. (a). Growth at 30° in the medium used for the incorporation experiments (see MATERIALS AND METHODS). Turbidity was followed by use of a Klett–Summerson colorimeter with a No. 54 filter. (b). Incorporation of [^{14}C]dT (8 μg/ml, 300 μC/mmole) into the acid-insoluble fraction at 30°; ●, wild-type; ○, mutant.

Fig. 2. Activities of dT phosphorylase and dT kinase in extracts of wild-type (W3110) and mutant (KY688) bacteria. (a). dT phosphorylase in crude extracts (protein, 200 μg). (b). dT kinase using [^{14}C]dT (360 μC/mmole) as a substrate in crude (400 μg protein) or heated (120 μg protein) extracts. (c). dT kinase using [^{3}H]dU (360 μC/mmole) as substrate in crude (400 μg protein) or heated (120 μg protein) extracts. Symbols: Crude (●) and heated (▲) extracts of wild-type cells. Crude (○) and heated (△) extracts of mutant cells.

Activities of dT phosphorylase and dT kinase

The activity of dT phosphorylase in crude extracts was first examined. As might have been expected, no appreciable difference in specific activity of this enzyme was detected between the wild-type and the mutant strains (Fig. 2a). The mutant strain used in this and the following experiment is KY688 which is a derivative of strain KY608, whose temperature sensitivity has been lost by reversion and an additional requirement for isoleucine and valine has been introduced.

Activity of dT kinase was then measured, and the results are shown in Figs. 2b and 2c. No measurable activity was observed either in the crude or heated extract of the mutant in these assays with dT or dU as a substrate, where 2 % of the wild-type activity would have been detected. In order to test whether the apparent loss of activity is due to the presence of an enzyme inhibitor in the mutant extract, mixtures of heated extracts of the mutant and the wild-type strains in several proportions were examined for enzyme activity. As shown in Fig. 3, neither inhibitory effects of the mutant extract nor stimulatory effects of the wild-type extract was observed under these conditions.

Products of the enzymatic reaction were then analyzed by paper chromatog-

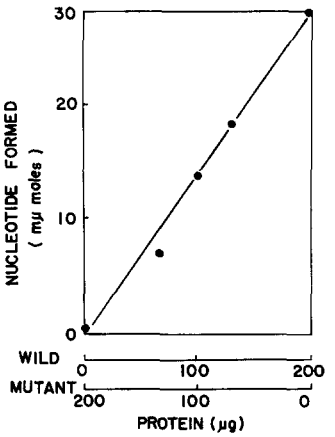

Fig. 3. Activities of dT kinase in the mixtures of heated extracts from wild type (W3110) and mutant (KY688). Reaction mixtures containing wild-type and mutant extracts in the proportions indicated were incubated for 60 min under otherwise standard conditions.

TABLE I

PRODUCTS OF THE dT-KINASE REACTION WITH CRUDE AND HEATED EXTRACTS OF WILD-TYPE (W3110) AND THE MUTANT (KY688)

The incubation mixture and the methods of analysis of the reaction products were described in MATERIALS AND METHODS. The reaction (0.9-ml scale) with either crude extract (protein, 1200 μg) or heated extract (protein, 350 μg) was carried out at 37° for 60 min. Figures represent per cent of the total radioactivity recovered after the first chromatography.

Products	*Zero time control*	*Wild-type extracts*		*Mutant extracts*	
		Crude	*Heated*	*Crude*	*Heated*
dT	99.5	1.9	3.1	2.6	99.5
Thymine	<0.2	49.7	<0.2	97.4	<0.2
dTMP	<0.2	35.4	94.1	<0.2	<0.2
dTDP	<0.2	11.5	2.7	<0.2	<0.2
dTTP	<0.2	1.5	<0.2	<0.2	<0.2
Total radioactivity (counts/min)	5929	6390	5556	9790	6192

raphy with the results shown in Table I. With the crude extract of the wild-type strain, about 50 % of dT was converted to dTMP or dTDP during the incubation, whereas no measurable radioactivity was observed in the nucleotide fractions with the mutant extract. Instead, most of the radioactivity was recovered as thymine, presumably due to the activity of dT phosphorylase. With heated extracts in which the latter activity had been inactivated, most of the [^{14}C]dT was converted to dTMP in the case of the wild type while it remained essentially unaltered in the case of the mutant.

These results seem to support the conclusion that the mutant is incapable of forming appreciable amounts of active dT kinase at least under the conditions employed. The data also suggest that dT kinase is perhaps the only enzyme respon-

sible for incorporating exogenously supplied dT into the acid-insoluble fraction in *E. coli* strain K12.

Incorporation of dBU in vivo

Since several 5-substituted deoxyuridine derivatives, including dBU, serve as substrates for dT kinase of *E. coli*[1], the mutant may also be expected to be unable to incorporate dBU, provided that the enzyme is solely responsible for the incorporation of dBU as well as dT into the acid-insoluble form. This could be tested conveniently by examining the sensitivity of cells that had been grown in the presence of dBU to near ultraviolet light, since it has been shown that *E. coli* cells that incorporate dBU become extremely sensitive to near ultraviolet light[13]. As shown in Fig. 4,

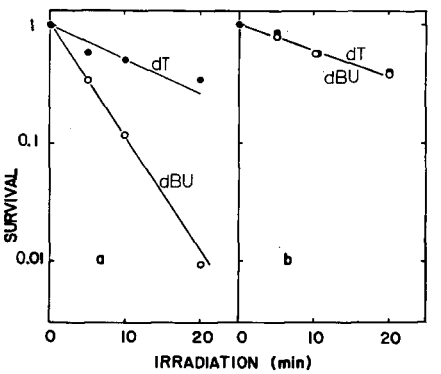

Fig. 4. Effect of near-ultraviolet light irradiation on wild-type and mutant cells grown in a medium containing dBU. Wild-type strain W3110 (a) and the mutant strain KY688 (b) were grown in the presence of dT (●) or dBU (○) and were irradiated under conditions described in MATERIALS AND METHODS.

wild-type cells grown in the presence of dBU became markedly sensitized as compared to those grown in the presence of dT, whereas the mutant remained resistant to the irradiation whether grown in the presence of dBU or dT. These results suggest that the mutant cells are virtually unable to incorporate dBU into DNA and that the incorporation of dBU in *E. coli* K12, like that of dT, depends mostly, if not completely, on the activity of dT kinase.

Sensitivity of the mutant to 5-fluorouracil (FU) or dFU

FU and dFU are well-known inhibitors of DNA and RNA synthesis in *E. coli* and their effects can be overcome only when excess of uridine and dT are added to the medium[14]. In other words, in the presence of FU or dFU, one can create conditions where DNA synthesis would depend on exogenously supplied dT. It would be expected therefore that the wild-type strain would be capable of growth on a peptone-glucose medium containing FU or dFU, uridine and dT, whereas the mutant would not, in view of the present results showing that dT kinase is mostly responsible for incorporation of dT. This expectation was borne out by experiments

in which washed cells of wild type or the mutant were either plated or streaked onto agar medium containing the three compounds listed above; only wild-type cells showed visible growth after 2 days incubation at 37°.

When mutant cells that had been treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine were plated on this medium, FU- or dFU-resistant 'revertants' were obtained. Although 20 such colonies were picked from the medium containing dFU, purified and examined for their ability to incorporate [^{14}C]dT, none had recovered such an ability, indicating that their capacity to grow in the presence of dFU was due to a mechanism other than the recovery of the dT-kinase activity. No further attempts were made to isolate true revertants.

dT kinase formed by infection of T even phage

When the mutant bacteria were infected with T even phage, incorporation of [^{14}C]dT into the acid-insoluble fraction occurred after about 10 min. The incorporation could be shown to take place after infection with phage T2 or T4D, but not with T1 or T5 (Fig. 5). The results suggest that the synthesis of dT kinase can be induced

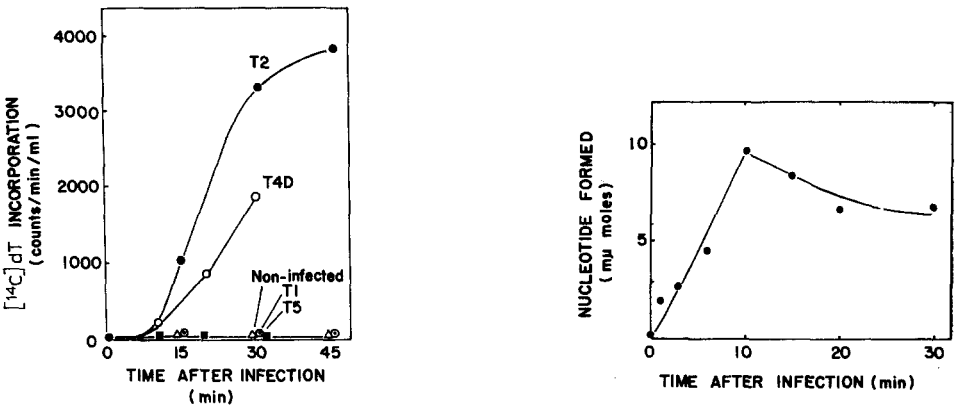

Fig. 5. Incorporation of [^{14}C]dT into the acid-insoluble fraction of the mutant bacteria (KY895) infected with phage T1, T2, T4D, and T5. Each phage was infected at a multiplicity of 5 to 10, and [^{14}C]dT (2 μg/ml, 1.2 mC/mmole) was added at the time of infection.

Fig. 6. Appearance of dT-kinase activity in mutant bacteria (KY895) after infection with phage T4D. Reaction mixture with [2-^{14}C]dT (280 μC/mmole) and extract (300 μg protein) that had been passed through Sephadex G-25 was incubated at 30° for 60 min under otherwise standard conditions.

by infection of *E. coli* with T2 or T4D phage. In fact, the activity of dT kinase was detected in crude extracts of mutant bacteria after infection with phage T4D (Fig. 6). The enzyme synthesis started immediately after infection and leveled off at around 10 min. This phage-induced enzyme required ATP, Mg^{2+} or Mn^{2+} for its activity and seemed to be inhibited by dTTP (Table II). In contrast to the bacterial dT kinase, however, the enzyme activity in phage-infected cells was found to be sensitive to heat treatment at 45° (Fig. 7). In a separate experiment in which a mixture of bacterial and T4 phage-induced dT kinase was heated, no evidence was obtained to

TABLE II

dT KINASE IN THE MUTANT BACTERIA INFECTED WITH PHAGE T4D

Bacteria (KY895) infected with T4D were harvested after 10 min. Reaction mixture containing [2-^{14}C]dT (280 μC/mmole) was incubated for 60 min at 30° or 37°.

Enzyme source		*System*	*Assay temperature*	*Nucleotide formed (mμmoles)*
KY895	Infected*	Complete	30	10.6
		+dTTP†	30	3.0
		$-MnCl_2$	30	7.0
		$-MgCl_2$	30	10.2
		$-MgCl_2$, $MnCl_2$	30	0.4
		−ATP	30	<0.2
		Complete	37	5.0
KY895	Non-infected*	Complete	30	<0.2
W3110	Non-infected	Complete**	37	46.8
		+dTTP†	37	1.0
		Complete***	37	45.6

* Sephadex G-25 treated extract (protein 200 μg).
** Crude extract (protein 400 μg).
*** Extract heated for 5 min at 70°.
† Addition of 350 mμmoles dTTP.

suggest the presence of a stabilizer in non-infected cell extracts or of a destabilizer in extracts of T4-infected cells. These results suggest that the dT-kinase activity which appears after infection with T4D phage is qualitatively different from that of the wild-type host bacteria.

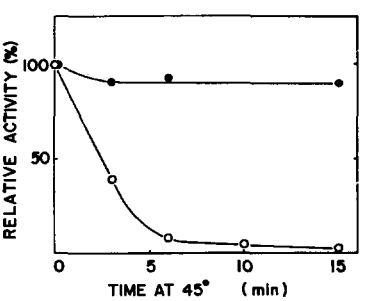

Fig. 7. Thermostability of dT kinase from mutant bacteria (KY895) infected with phage T4D and of the enzyme from non-infected wild-type cells (W3110). Sephadex-treated extracts of T4D-infected mutant cells (200 μg, ○), and of non-infected wild-type cells (200 μg, ●) were heated at 45° as indicated and were incubated with other components of the reaction mixture for 60 min at 30°.

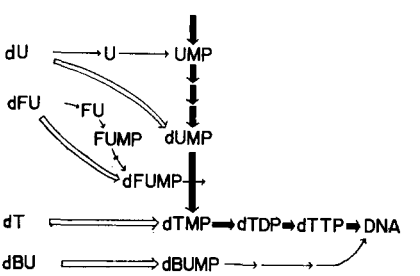

Fig. 8. A partial pathway of deoxyribonucleotides and DNA synthesis in *E. coli*. Symbols: Black arrows, the main pathway of DNA synthesis; open arrows, reactions catalyzed by dT kinase.

DISCUSSION

The data presented above lead to the conclusion that the mutant strain cannot incorporate external [^{14}C]dT into DNA due to the lack of dT-kinase activity. The mutant must be able to synthesize dTMP endogenously since its growth is normal in a minimal medium with or without dT. The dT kinase of wild-type *E. coli* can catalyze the conversions of dT, dU, dBU, and dFU to their corresponding nucleotides[1] as shown schematically in Fig. 8. Extracts of the mutant failed to catalyze the conversion of dT and dU to their nucleotides (Figs. 2b and 2c), and the mutant also could not incorporate dBU into DNA *in vivo* as judged by the lack of sensitization to near ultraviolet light (Fig. 4). It thus seems that the activity of dT kinase with most, if not all, of the known substrates is lacking in this mutant.

It has been reported that FU and its derivatives inhibit at least 3 different processes in *E. coli*, *i.e.* the syntheses of DNA, RNA, and the cell wall, thus resulting in the cessation of growth[14]. The inhibitory effects of FU or dFU could be overcome by additions of excessive amounts of uridine and dT in the wild-type strain, but not in the mutant. The mutant cells elongated to form filaments and lost their colony-forming ability under these conditions. Since 5-fluorodeoxyuridine (dFUMP) is known to be a potent inhibitor of thymidylate synthetase, that catalyzes the conversion of dUMP into dTMP[15], the present results lead to the conclusion that thymidylate synthesis by the latter enzyme is blocked in both wild-type and mutant bacteria, even in the presence of dT and uridine in the medium, and that dTMP synthesis must depend on externally supplied dT.

Then the question might arise as to the mode of conversion of FU or dFU, particularly in the dT kinase-deficient mutant. The direct conversion of dFU to dFUMP by dT kinase presumably does not operate at least in the mutant, and it seems most probable that the pathway dFU → FU → FUMP → dFUMP (see Fig. 8) is operative since enzymes are known in *E. coli* that catalyze the series of reactions involved in this pathway. Indeed, the parallel reactions dU → U → UMP → dUMP were found to take place in the mutant as well as in wild-type bacteria as judged by the incorporation of [^{3}H]dU into both DNA and RNA.

Genetic studies[16] revealed that the dT kinase-deficient mutant used in this study is due to a mutation of a single gene (designated as *tdk*) located close to att_{80} on the opposite side from the *trp* operon on the *E. coli* chromosome. The *tdk*$^+$ marker was shown to be co-transduced with *ton*, *trp*, and *cysB* markers into *tdk–1* mutant bacteria by phage P1, and also transduced by phage φ80. The heterogenote that carries normal φ80 and defective φ80*dtdk*$^+$ and is phenotypically *Tdk*$^+$ has thus been isolated. Upon induction of such heterogenotes with mitomycin C, the specific activity of dT kinase increased more than several fold. Moreover, when *tdk–1* mutant bacteria were infected with phage lysates from induced heterogenotes, the activity of dT kinase appeared almost instantaneously. These results suggest that the *tdk–1* gene represents the structural gene for this enzyme rather than a gene exerting some regulatory or other indirect functions affecting the synthesis or activity of dT kinase, although the latter possibilities are not ruled out at the present time.

It is possible that additional mutants affecting dT-kinase activity could be selectively screened by making use of the resistance of such mutants, compared to the wild type, to near ultraviolet irradiation after growth in a medium containing

dBU. In fact, several mutants that are unable to grow on a peptone–glucose medium containing FU, uridine and dT have been isolated by this method[16].

The synthesis of a number of enzymes related to DNA synthesis is known to be induced when *E. coli* cells are infected with phage T2 or T4 (ref. 17), and the phage genes carrying structural information of some of these enzymes have been identified[18–20]. The present results show that the activity of phage-induced dT kinase also appears immediately when cells are infected with T4D (Fig. 6). The enzyme from phage-infected cells was found to be very sensitive to heat, unlike the host bacterial enzyme (Fig. 7). This agrees well with the results obtained with wild-type *E. coli* strain B that had been infected with an amber mutant (*am82*) of phage T4 (R. OKAZAKI, personal communication). These findings suggest that a phage-specific dT kinase is induced by T4 phage infection, and the enzyme represents another example of "early enzyme".

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