Thymidine Kinase of Bacteria: Activity of the Enzyme in Actinomycetes and Related Organisms

By HAJIME SAITO* AND HARUAKI TOMIOKA

Department of Microbiology and Immunology, Shimane Medical University, Izumo 693, Japan

(Received 11 October 1983; revised 14 March 1984)

Various micro-organisms were studied for their thymidine kinase (adenosine 5'-triphosphate:thymidine 5'-phosphotransferase, EC 2.7.1.21) (TK) activity. The sonicated cell extract of *Escherichia coli* K12 had a TK activity of 35–66 pmol thymidine monophosphate formed min^{-1} (mg protein) $^{-1}$. The cell extracts of *Salmonella typhimurium* and *Klebsiella pneumoniae* showed a markedly higher (5- to 11-fold) TK activity. Somewhat lower but significant TK activity was detected in the cell extracts of *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis* and *Proteus mirabilis*. In contrast, weak TK activity, if any, was detected in the cell extracts of *Pseudomonas aeruginosa*. This was also the case with respect to the cell extracts of various actinomycetes (such as *Nocardia* and *Streptomyces*) and related organisms (such as *Corynebacterium*, *Mycobacterium* and *Rhodococcus*).

INTRODUCTION

Thymidine kinase (TK), one of the key enzymes in the pyrimidine salvage pathway, catalyses the phosphorylation of thymidine (TdR) to thymidine 5'-monophosphate (TMP) (Okazaki & Kornberg, 1964*a*; Ellims & Van Der Weyden, 1981). The physiological importance of TK is indicated by its high activity in cells which engage in active DNA synthesis (Hotta & Stern, 1963) and by the evolution of an intricate feedback system to regulate it (Okazaki & Kornberg, 1964*b*). The physiological function of TK is also evident because TMP is able to correct its deficiency in cells which lack thymidylate synthetase or in cells in which the synthetase is inhibited for some reason. With respect to the TK of micro-organisms, however, only a few studies have been reported: TK from *Escherichia coli* (Okazaki & Kornberg, 1964*a, b*), *Bacillus megaterium* (Wachsman & Morgan, 1971), *Mycoplasma hominis* (Stock & Gentry, 1971), and *Dictyostelium discoideum* (Michrina & Deering, 1980). The precise pattern of TK distribution among various micro-organisms has not been documented. Grivell & Jackson (1968) reported the lack of TK in some ascomycetes such as *Neurospora crassa*, *Aspergillus nidulans*, *Saccharomyces cerevisiae* and *Euglena gracilis*, and Mizuno (1969) found that *Pseudomonas schuylkilliensis* also lacks TK.

In a preliminary experiment, we found that rapidly growing mycobacteria did not efficiently incorporate [^3H]TdR into their DNA. This was not overcome by 5-fluorodeoxyuridine (FUDR), which is converted to 5-fluorodeoxyuridylylate *in vivo* and inhibits thymidylate synthetase, a key enzyme of *de novo* deoxythymidine triphosphate (TTP) synthesis (Mathews & Cohen, 1963). Deoxyadenosine (AdR) did not lead to an uptake of [^3H]TdR to DNA by these mycobacteria. Because the *de novo* pathway of TTP synthesis is assumed to suppress the TK activity in the salvage pathway and because AdR inhibits TdR degradation by depressing TdR phosphorylase activity (Yagil & Rosner, 1970), the rapidly growing mycobacteria seemed to lack TK activity. This finding led us to study the precise distribution pattern of TK in various micro-organisms, especially in mycobacteria and related organisms. It is of interest to know whether or not these microbes can produce intrinsic TK, which determines their ability to use a scavenger pathway to supply TMP (and finally TTP) for DNA synthesis.

Abbreviations: AdR, deoxyadenosine; FUDR, 5-fluorodeoxyuridine; TdR, thymidine; TK, thymidine kinase; TMP, thymidine monophosphate; TTP, deoxythymidine triphosphate.

METHODS

Organisms. The following organisms were used: *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Corynebacterium cystitidis*, *C. pilosum*, *C. renale*, *Streptomyces roseoviridis*, *S. lavendulae*, *S. platensis*, *S. violascens*, *Nocardia asteroides*, *N. brasiliensis*, *Rhodococcus aurantiacus*, *Mycobacterium tuberculosis*, *M. bovis*, *M. kansasii*, *M. marinum*, *M. goodii*, *M. scrofulaceum*, *M. intracellulare*, *M. nonchromogenicum*, *M. terrae*, *M. gastri*, *M. fortuitum*, *M. chelonae* (*chelonae*) subsp. *abscessus*, *M. smegmatis*, *M. phlei*, *M. chitae*, *M. parafortuitum* and *M. diernhoferi*. Strain numbers are given in the relevant tables.

Chemicals. The following radioactive chemicals were purchased from New England Nuclear: [^3H]leucine, [^3H]uridine, [^3H]adenine and [^3H]thymidine. Deoxyadenosine and adenosine 5'-triphosphate were obtained from P-L Biochemicals, Milwaukee, USA and Oriental Yeast Co., Osaka, Japan, respectively. Other special agents were purchased from Wako Pure Chemical Inc., Osaka, Japan.

Assay for leucine, uridine, adenine and TdR incorporation. Some rapidly growing mycobacteria and *E. coli* K12 were cultured in heart infusion broth (Eiken Chemicals Co., Tokyo, Japan) and in the same broth containing 4% glycerol with 0.1% (v/v) Tween 80 at 37 °C for 2 d with shaking. The organisms were harvested and then suspended in the corresponding fresh medium at an optical density at 540 nm of 1 or 2. After addition of [^3H]leucine (5 Ci mol $^{-1}$, 2 $\mu\text{Ci ml}^{-1}$; 1 Ci = 37 GBq), [^3H]uridine (5 Ci mol $^{-1}$, 2 $\mu\text{Ci ml}^{-1}$), [^3H]adenine (1 Ci mol $^{-1}$, 2 $\mu\text{Ci ml}^{-1}$), or [^3H]TdR (5 Ci mol $^{-1}$, 1 or 2 $\mu\text{Ci ml}^{-1}$), cultivation was done at 37 °C with reciprocal shaking at 90 cycles min $^{-1}$ for up to 4 h. At various intervals, samples of culture (1 ml) were withdrawn, mixed with 1 ml cold 20% (w/v) trichloroacetic acid (TCA), and the resultant precipitate was collected on a Whatman GFC glass fibre filter. After washing with 5% TCA and then ethanol, the filter was dried and counted in a Packard Tri-Carb scintillation counter using toluene-based scintillation fluid. [^3H]Adenine incorporation into DNA was assayed as follows. Samples of culture (1 ml) were withdrawn, mixed with 2 ml 1 M-KOH, left to stand overnight at room temperature to hydrolyse RNA, and then mixed with 1.5 ml 50% (w/v) TCA. The resultant precipitates were collected on glass fibre filters, washed with 5% TCA and the ethanol and radioactivity were counted.

Preparation of cell extracts. Bacteria were cultured with shaking in heart infusion broth at 37 °C for 1 to 2 d, except for *Streptococcus pyogenes* which was cultured in heart infusion broth supplemented with 10% (v/v) foetal bovine serum. Other organisms such as actinomycetes and related organisms were cultured with shaking usually at 37 °C using the following media: *Corynebacterium*, heart infusion broth (2 d) (Guirard & Snell, 1981); *Streptomyces*, soluble starch/yeast extract medium (30 °C for 5 to 7 d) (Liu *et al.*, 1977); *Nocardia* and *Rhodococcus*, heart infusion broth containing 4% (v/v) glycerol (2 d) (Goodfellow, 1971); slowly growing mycobacteria, Dubos medium (Eiken; 10 to 12 d, *M. marinum* was grown at 33 °C) (Wayne *et al.*, 1964); rapidly growing mycobacteria, Dubos medium (Wayne *et al.*, 1964) or heart infusion broth containing 4% (v/v) glycerol and 0.1% Tween 80 (2 d) (Takeya *et al.*, 1978). Bacteria of each strain were harvested by centrifugation at 10000 g for 10 min, washed once with 50 mM-Tris/HCl buffer (pH 8.0) containing 1 mM each of dithiothreitol (DTT) and EDTA and suspended in the same buffer at a concentration of 0.05 to 0.25 g wet weight ml $^{-1}$. The bacterial suspension was then sonicated using an ultrasonic disintegrator (Insonator, Model 200M; Kubota Shoji, Tokyo, Japan) at 200 W for 5 min followed by centrifugation at 20000 g for 30 min. The supernatant was used as cell extract.

Assay for TK activity. Measurement of TK activity of cell extracts was done according to the method of Kizer & Holman (1974) with modifications. Briefly, the reaction mixture (0.2 ml) consisted of 62.5 mM-Tris/HCl (pH 8.0), 10 mM-ATP, 10 mM-MgCl $_2$, 2.5 mM-NaF, 1 mM-DTT, 250 μg bovine serum albumin ml $^{-1}$ (as a stabilizer of TK) (Okazaki & Kornberg, 1964a), 10 μM -[^3H]TdR (500 Ci mol $^{-1}$), and cell extract (50 or 100 μl). The reaction mixture was incubated with shaking at 100 cycles min $^{-1}$ at 37 °C for up to 120 min. After incubation, the mixture was boiled for 3 min and a 0.1 ml sample was dispensed on a DEAE-cellulose paper (2 $\times$ 2.1 cm; Tohyo Roshi Co., Tokyo, Japan). After drying at 90 °C for 5 min, the paper was rinsed in 1 mM-ammonium formate and then ethanol, dried at 120 °C and the radioactivity was counted in a liquid scintillation counter using toluene-based scintillation fluid. One unit of TK activity was defined as the amount of enzyme which generated 1 nmol TMP per 60 min.

RESULTS

Indirect evidence for TK deficiency in rapidly growing mycobacteria

Incorporation of TdR into high molecular weight cell components of *M. diernhoferi* ATCC 19340 was markedly lower than incorporation of leucine and uridine (Fig. 1a). Since the incorporation of leucine and uridine were specifically inhibited by streptomycin and by rifampicin, respectively, most of the [^3H]leucine and [^3H]uridine seemed to be incorporated into protein and RNA. However, the [^3H]TdR uptake by *M. diernhoferi* was not affected by

Fig. 1. Incorporation rates of labelled leucine, uridine, adenine and TdR into high molecular weight components (a) and DNA (b) of *Mycobacterium diernhoferi* ATCC 19340. (a) $\circ$, $[^3\text{H}]$ Leucine; $\bullet$, $[^3\text{H}]$ leucine + streptomycin ($200 \mu\text{g ml}^{-1}$); $\triangle$, $[^3\text{H}]$ uridine; $\blacktriangle$, $[^3\text{H}]$ uridine + rifampicin ($100 \mu\text{g ml}^{-1}$); $\square$, $[^3\text{H}]$ TdR; $\blacksquare$, $[^3\text{H}]$ TdR + nalidixic acid ($50 \mu\text{g ml}^{-1}$). (b) $[^3\text{H}]$ Adenine (AR) incorporation into DNA fraction.

Fig. 2. Incorporation of $[^3\text{H}]$ TdR into high molecular weight components of *E. coli* K12 and some rapidly growing mycobacteria in the absence (a) or presence (b) of $100 \mu\text{M}$ -FUdR. $\circ$, *E. coli* K12; $\triangle$, *M. chelonae* ATCC 14472; $\square$, *M. fortuitum* ATCC 6841; $\bullet$, *M. smegmatis* ATCC 14468; $\blacktriangle$, *M. phlei* ATCC 19249; $\blacksquare$, *M. chitae* ATCC 19627; $\bullet$, *M. parafortuitum* ATCC 19686; Δ , *M. diernhoferi* ATCC 19340.

nalidixic acid, indicating that this small $[^3\text{H}]$ TdR uptake was independent of DNA synthesis. The incorporation of adenine into the DNA fraction of *M. diernhoferi* was comparable with that of leucine and of uridine (Fig. 1b). Incorporation of $[^3\text{H}]$ TdR by other rapidly growing mycobacteria such as *M. fortuitum*, *M. chelonae*, *M. smegmatis*, *M. chitae*, *M. parafortuitum* and *M. phlei* was also markedly lower than that of *E. coli* K12 (Fig. 2a). When FUdR was added to the culture medium to prevent *de novo* synthesis of TMP by inhibiting thymidylate synthetase (Mathews & Cohen, 1963), incorporation of $[^3\text{H}]$ TdR by *E. coli* was remarkably accelerated, but

this was not the case for uptake of [^3H]TdR by rapidly growing mycobacteria (Fig. 2*b*). A separate experiment showed that FdR at a high concentration (600 μM) and a combination of 100 μM -FdR with 60 mM-AdR failed to augment incorporation of [^3H]TdR by *M. diernhoferi*. This indicates a TK deficiency in rapidly growing mycobacteria, because, if a given organism had a substantial amount of TK, the combination of FdR with AdR should enhance incorporation of TdR into DNA for the following reasons. Firstly, FdR suppresses *de novo* TMP synthesis resulting in a recovery of the TK activity from the suppressed state mediated by the *de novo* TMP synthesis pathway; and secondly, AdR suppresses degradation of TdR by inhibiting TdR phosphorylase (Yagil & Rosner, 1970).

Lack of TK activity in cell extracts of various mycobacteria

Since the results described above strongly suggested that rapidly growing mycobacteria lacked TK activity, the cell extracts of various mycobacteria were assayed directly for TK activities. Fourteen strains of the seven species of rapidly growing mycobacteria tested showed no significant TK activity in their cell extracts when they were cultured in Dubos medium (Table 1). Similar TK deficiency was also observed when they were cultivated in heart infusion broth containing glycerol and Tween 80 (data not shown). Table 1 also shows that the cell

Table 1. *TK activity of various mycobacteria*

Cell extracts of mycobacteria were tested for TK activity at the following protein concentrations in the reaction mixture: rapid growers, 120 to 200 $\mu\text{g ml}^{-1}$; slow growers, 50 to 110 $\mu\text{g ml}^{-1}$.

Strain*	Relative specific TK activity in cell extract (%)†	Relative TK production per g cell (%)†
Rapidly growing mycobacteria		
<i>M. fortuitum</i> ATCC 6841 ^b	0.27	0.03
<i>M. fortuitum</i> ATCC 23012 ^a	0	0
<i>M. chelonae</i> ATCC 14472 ^b	0	0
<i>M. chelonae</i> Yamamoto ^c	0	0
<i>M. smegmatis</i> ATCC 14468 ^b	1.00	0.03
<i>M. smegmatis</i> ATCC 19979 ^b	0	0
<i>M. phlei</i> ATCC 19249 ^b	0	0
<i>M. phlei</i> WA-289 ^a	0.09	0.01
<i>M. chitae</i> ATCC 19627 ^b	0	0
<i>M. chitae</i> ATCC 19629 ^b	0	0
<i>M. parafortuitum</i> ATCC 19686 ^b	0	0
<i>M. parafortuitum</i> ATCC 19687 ^b	0	0
<i>M. diernhoferi</i> ATCC 19340 ^b	0	0
<i>M. diernhoferi</i> SN1412 ^a	0	0
Slowly growing mycobacteria		
<i>M. tuberculosis</i> H37Rv ^f	1.93	0.08
<i>M. bovis</i> BCG ^g	0	0
<i>M. kansasii</i> ATCC 12478 ^a	0	0
<i>M. marinum</i> Kohmoto ⁱ	0	0
<i>M. gordonae</i> ATCC 14470 ^e	0	0
<i>M. scrofulaceum</i> ATCC 15978 ^e	0	0
<i>M. intracellulare</i> ATCC 15985 ^b	0	0
<i>M. nonchromogenicum</i> ATCC 19530 ^e	8.13	0.44
<i>M. terrae</i> ATCC 15755 ^e	0	0
<i>M. gastri</i> ATCC 15754 ^h	0	0

* Origins of the strains are as follows: *a*, Communicable Disease Center, Atlanta, Ga., USA; *b*, American Type Culture Collection, Rockville, Md., USA; *c*, K. Urabe, Hiroshima University, Hiroshima, Japan; *d*, R. Bonicke, Forschungsinstitut Borstel, Borstel, FRG; *e*, M. Tsukamura, National Sanatorium, Chubu Hospital, Aichi, Japan; *f*, H. Shoji, Osaka University, Osaka, Japan; *g*, Nihon BCG Seizo Co., Tokyo, Japan; *h*, L. G. Wayne, Veterans Administration Hospital, San Fernando, Calif., USA; *i*, clinical isolate by H. Saito.

† The value for *E. coli* K12 grown in the same medium as used for these mycobacteria (Dubos medium) was taken as 100%. Specific activity and production of TK in *E. coli* K12 were 66 pmol min⁻¹ mg⁻¹ and 280 units per g cell, respectively.

extracts from slowly growing mycobacteria grown in Dubos medium lacked TK activity. Separate experiments showed that these mycobacteria grown in Kirchner's medium did not contain any significant amount of TK either (unpublished observation).

Table 2. TK activity of actinomycetes and related organisms

Cell extracts of the organisms were examined for TK activity at a protein concentration of 200 $\mu\text{g ml}^{-1}$ in the reaction mixture except for *Nocardia* (500 $\mu\text{g ml}^{-1}$).

Strain*	Relative specific TK activity in cell extract (%)†	Relative TK production per g cell (%)†
<i>Nocardia asteroides</i> ATCC 23824	0	0
<i>Nocardia asteroides</i> R860	0.17	0.05
<i>Nocardia asteroides</i> ATCC 19247	0.56	0.19
<i>Nocardia brasiliensis</i> R432	0.30	0.04
<i>Nocardia brasiliensis</i> R887	0.01	0
<i>Rhodococcus aurantiacus</i> ATCC 25938	0	0
<i>Rhodococcus aurantiacus</i> E7973	0	0
<i>Rhodococcus aurantiacus</i> E8182	0	0
<i>Streptomyces roseoviridis</i> H646SY3	0	0
<i>Streptomyces lavendulae</i> H698SY2	0	0
<i>Streptomyces platensis</i> H273SY2	0	0
<i>Streptomyces violascens</i> H82NSY7	0	0
<i>Corynebacterium cystitidis</i> D454	10.3	3.23
<i>Corynebacterium pilosum</i> 46HARA	0	0
<i>Corynebacterium renale</i> 65-122	0	0

* Origins of the strains are as follows: *Nocardia* and *Rhodococcus*, M. Tsukamura, National Sanatorium, Chubu Hospital, Aichi, Japan; *Streptomyces*, S. Nakamura, Hiroshima University, Hiroshima, Japan; *Corynebacterium*, T. Hiramine, College for Dairy Farming, Hokkaido, Japan.

† The value for *E. coli* K12 grown in the same media as used for these organisms was taken as 100%. Specific TK activity ($\text{pmol min}^{-1} \text{mg}^{-1}$) and TK production (units per g cell) of *E. coli* K12 grown in various media were, respectively, as follows: heart infusion broth (for *Corynebacterium*), 35.2 and 226; heart infusion broth containing 4% glycerol (for *Nocardia* and *Rhodococcus*), 34.8 and 174; soluble starch/yeast extract medium (for *Streptomyces*), 21.2 and 108.

Table 3. TK activity of bacteria

Cell extracts of the bacteria were examined for TK activity at a protein concentration of 50 $\mu\text{g ml}^{-1}$ in the reaction mixture, except for *P. aeruginosa* PAO3047, NC-5 and 584A43 (2.5 mg ml^{-1}).

Strain*	Relative specific TK activity in cell extract (%)†	Relative TK production per g cell (%)†
<i>Staphylococcus aureus</i> 209P ^a	83	59
<i>Streptococcus pyogenes</i> ST1 ^b	29	4.6
<i>Bacillus subtilis</i> B7 ^b	13	1.8
<i>Salmonella typhimurium</i> HKB-1 ^d	1130	878
<i>Escherichia coli</i> K12 ^c	100	100
<i>Klebsiella pneumoniae</i> Kasuya ^c	528	329
<i>Proteus mirabilis</i> IFO3849 ^c	34	39
<i>Pseudomonas aeruginosa</i> HKB-2 ^d	0	0
<i>Pseudomonas aeruginosa</i> PAO3047 ^e	0	0
<i>Pseudomonas aeruginosa</i> NC-5 ^f	0.2	0.3
<i>Pseudomonas aeruginosa</i> 584A43 ^g	0	0

* Origins of the strains are as follows: a, Y. Kanemasa, Okayama University, Okayama, Japan; b, A. Takagi, Tottori University, Tottori, Japan; c, A. Matsumae, Kitasato Institute, Tokyo, Japan; d, M. Kuwabara, Hiroshima Prefectural Hospital, Hiroshima, Japan; e, M. Kageyama, Mitsubishi Kasei, Institute for Life Science, Tokyo, Japan; f, S. Gotoh, Toho University, Tokyo, Japan; g, K. Okuzumi, Tokyo University, Tokyo, Japan.

† The value for *E. coli* K12 was taken as 100%. Specific activity and production of TK in *E. coli* K12 were 48.7 $\text{pmol min}^{-1} \text{mg}^{-1}$ and 147 units per g cell, respectively.

Table 4. *Effects of cell extracts from various micro-organisms on TK activity of E. coli K12*

Cell extracts of the organisms were tested for their effects on TK activity of the cell extract from *E. coli* K12 under the standard reaction conditions (see Methods). TK activity of *E. coli* K12 cell extract was measured at a protein concentration of $250 \mu\text{g ml}^{-1}$ in the reaction mixture with or without the addition of the other cell extracts ($250 \mu\text{g protein ml}^{-1}$).

Cell extract from:	TMP generation (nmol min ⁻¹)*	Inhibition (%)
None	575	0
<i>Corynebacterium pilosum</i> 46HARA	389	32
<i>Streptomyces violascens</i> H82NSY7	147	74
<i>Nocardia asteroides</i> ATCC 23824	471	18
<i>Nocardia brasiliensis</i> R432	490	15
<i>Rhodococcus aurantiacus</i> ATCC 25938	432	25
<i>Mycobacterium intracellulare</i> ATCC 15985	537	7
<i>Mycobacterium kansasii</i> ATCC 12478	315	45
<i>Mycobacterium chelonae</i> Yamamoto	469	18
<i>Mycobacterium fortuitum</i> ATCC 6841	137	76
<i>Mycobacterium smegmatis</i> ATCC 14468	456	21
<i>Mycobacterium phlei</i> ATCC 19249	412	28

* The amount of TMP generation in the reaction mixture (0.2 ml).

TK deficiency in mycobacteria-related organisms

Table 2 shows TK activity of the cell extracts of the strains of *Corynebacterium*, *Streptomyces*, *Nocardia* and '*Gordona*'. All the organisms tested were essentially free from TK activity, except *C. cystitidis* which contained a low but significant amount of TK. Thus, the TK deficiency seemed to be a common feature of actinomycetes and related organisms. Table 3 shows the distribution of TK among common Gram-positive and Gram-negative bacteria. The Gram-positive cocci and rods, such as *Staphylococcus aureus*, *Streptococcus pyogenes* and *B. subtilis* had a comparable but lower amount of cell-associated TK than did *E. coli* K12. *Enterobacteriaceae* had a markedly high TK activity: *Salmonella typhimurium* and *Klebsiella pneumoniae* had 5- to 11-fold high TK activity than *E. coli* K12. The TK activity of *Proteus mirabilis* was somewhat lower than that of *E. coli* K12. As in the case of *Pseudomonas schuylkilliensis* (Mizuno, 1969), *P. aeruginosa* lacked TK activity.

Effects of cell extracts of some actinomycetes and related organisms on TK activity of E. coli K12

Representative cell extracts of actinomycetes and related organisms lacking TK activity were examined for their effects on the TK activity of the cell extract of *E. coli* K12 under the standard reaction conditions. Most cell extracts had a weak inhibitory activity against the enzyme action by TK of *E. coli* K12 (6 to 32% inhibition; Table 4), whereas the extracts of *Streptomyces violascens*, *Mycobacterium kansasii* and *M. fortuitum* showed a somewhat greater inhibition.

DISCUSSION

We examined TK activity in cell extracts of various organisms. In most of the actinomycetes and related organisms studied TK activity was undetectable by our assay system. This does not appear to be due to the different physiological state of test organisms from that of *E. coli* K12, for the following reasons. Firstly, the TK activity of a given organism was comparable to that of *E. coli* K12 grown in the same medium and secondly, *Corynebacterium pilosum*, *C. renale*, *Nocardia asteroides*, *N. brasiliensis*, *Rhodococcus aurantiacus* and various rapidly growing mycobacteria lacked cell-associated TK activity, although the cultivation time for them was the same as that for *E. coli* K12 (2 d). These examples suggest that the apparent TK deficiency in actinomycetes and related organisms is not due to a prolonged cultivation which may cause an increase in the ratio of physiologically inactive cells. Thus, it seems that actinomycetes and related organisms have an inherent TK deficiency. It was also found that *Pseudomonas aeruginosa* (24 h

cultivation) lacked cell-associated TK activity. Preliminary experiments showed that all the cultured cells of *P. aeruginosa* harvested at 6, 12, 24, 48 or 72 h had little, if any, TK activity (unpublished observation). Thus *P. aeruginosa* seems to lack the ability to produce TK.

When representative cell extracts of actinomycetes and related organisms were tested for their effects on TK activity of cell extract from *E. coli* K12, many were only weakly inhibitory although others showed definite inhibition (45 to 76%: Table 4). The inhibitory factor in the cell extracts of these microbes may be an active inhibitor directed against TK itself (Okazaki & Kornberg, 1964*b*; Kizer & Holman, 1974; Madhav *et al.*, 1980), TMP phosphatase, or TdR-decomposing enzymes such as TdR phosphorylase. The inhibitor may be an ATP phosphatase which stimulates TdR degradation in the reaction mixture by lowering the concentration of ATP (this antagonizes TdR degradation) as reported by Grivell & Jackson (1968). In any case, the inhibitory activity in the cell extracts of these organisms is too low to cause a complete lack of TK activity. Since the cell extracts were stored at -20 or -80 °C until use and the reaction mixture contained a sufficient amount of bovine serum albumin as TK stabilizer (Okazaki & Kornberg, 1964*a*; Kizer & Holman, 1974), the possibility can be excluded that the TK of these organisms was inactivated during their storage or enzyme assay incubation, although TK of *E. coli* and mammalian origin is unstable (Okazaki & Kornberg, 1964*a*; Hopgood & Ballard, 1974; Kizer & Holman, 1974; Ellims & Van Der Weyden, 1980).

The present findings strongly suggest an inherent deficiency of TK in the actinomycetes (*Nocardia* and *Streptomyces*) and related organisms (*Mycobacterium*, *Rhodococcus* and *Corynebacterium*). Preliminary experiments have also shown a similar TK deficiency in *Propionibacterium* and *Bifidobacterium* (unpublished observations). It has been reported that *Neurospora crassa*, *Saccharomyces cerevisiae*, *Aspergillus nidulans* and *Euglena gracilis* (Grivell & Jackson, 1968) and *Chlamydia psittaci* (Hatch, 1976) also lack TK. Our preliminary experiments have shown that *Candida* and *Cryptococcus* also lack TK (unpublished observations). Thus, it seems that TK deficiency is a general property of actinomycetes, fungi and related micro-organisms. Since TK is a key enzyme in the salvage pathway for TMP synthesis, the TK deficiency indicates that these organisms cannot use extracellular TdR, which is generated by DNA decomposition due to the action of extracellular deoxyribonuclease, to supply TTP for their DNA synthesis. It is of interest to know what kind of advantage and/or disadvantage is given to these taxonomically related micro-organisms by being deprived of their TK-producing ability. It is also of interest to know why *Pseudomonas aeruginosa* acquired the phenotype (genotype) of TK deficiency in its evolution, for the following reasons. This microbe grows rapidly in comparison to other bacteria. It is disadvantageous for such a rapidly growing organism to lack an inherent TK, since the salvage pathway for TMP (finally TTP) synthesis catalysed by TK has much higher activity than that of the *de novo* pathway, particularly in supplying TTP for active DNA synthesis (Hotta & Stern, 1963; Okazaki & Kornberg, 1964*a, b*). A study to elucidate the precise meaning of the TK deficiency in these organisms is now under way.

We thank the following investigators for providing cultures: M. Tsukamura, National Sanatorium, Chubu Hospital, Aichi; S. Nakamura, Hiroshima University, Hiroshima; T. Hiramine, College for Dairy Farming, Hokkaido, Japan. We also thank M. Ohara for reading this manuscript.

REFERENCES

- ELLIMS, P. H. & VAN DER WEYDEN, M. B. (1980). Human liver thymidine kinase. Purification and some properties of the enzyme. *Journal of Biological Chemistry* **255**, 11290–11295.
- ELLIMS, P. H. & VAN DER WEYDEN, M. B. (1981). Kinetic mechanism and inhibition of human liver thymidine kinase. *Biochimica et biophysica acta* **660**, 238–242.
- GOODFELLOW, M. (1971). Numerical taxonomy of some nocardioform bacteria. *Journal of General Microbiology* **69**, 33–80.
- GRIVELL, A. R. & JACKSON, J. F. (1968). Thymidine kinase: evidence for its absence from *Neurospora crassa* and some other micro-organisms, and the relevance of this to the specific labelling of deoxyribonucleic acid. *Journal of General Microbiology* **54**, 307–317.
- GUIRARD, B. M. & SNELL, E. E. (1981). Biochemical factors in growth. In *Manual of Methods For General Bacteriology*, pp. 79–111. Edited by P. Gerhardt, R. G. E. Murray, R. N. Costilow, E. W. Nester, W. A. Wood, N. R. Krieg & G. B. Phillips. Washington: American Society for Microbiology.
- HATCH, T. P. (1976). Utilization of exogenous thymi-

- dine by *Chlamydia psittaci* growing in thymidine kinase-containing and thymidine kinase-deficient L cells. *Journal of Bacteriology* **125**, 706–712.
- HOTTA, Y. & STERN, H. (1963). Molecular factors of mitotic regulation, I. Synthesis of thymidine kinase. *Proceedings of the National Academy of Sciences of the United States of America* **49**, 648–654.
- HOPGOOD, M. F. & BALLARD, F. J. (1974). The relative stability of liver cytosol enzymes incubated *in vitro*. *Biochemical Journal* **144**, 371–376.
- KIZER, D. E. & HOLMAN, L. (1974). Purification and properties of thymidine kinase from generating rat liver. *Biochimica et biophysica acta* **350**, 193–200.
- LIU, C., HERMANN, T. & MILLER, P. A. (1977). Feedback inhibition of the synthesis of an antibiotic: Aurodox (X-5108). *Journal of Antibiotics* **30**, 244–251.
- MADHAV, R., COETZEE, M. L. & OVE, P. (1980). Purification of thymidine kinase by affinity chromatography with an enzyme inhibitor as the ligand. *Archives of Biochemistry and Biophysics* **200**, 99–107.
- MATHEWS, C. K. & COHEN, S. S. (1963). Inhibition of phage-induced thymidylate synthetase by 5-fluoro-deoxyuridylate. *Journal of Biological Chemistry* **238**, 367–370.
- MICHRINA, C. A. & DEERING, R. A. (1980). Thymidine kinase activity in *Dictyostelium discoideum*. *Journal of General Microbiology* **119**, 263–266.
- MIZUNO, S. (1969). Fractionation of nucleic acids. In *Methodology for Biochemistry: [A-2] Separation, Purification, and Measurement of Nucleic Acids*, pp. 16–67. Edited by I. Uritani, H. Shimura, M. Nakamura & M. Funazu. Tokyo: Tokyo University Press.
- OKAZAKI, R. & KORNBERG, A. (1964a). Deoxy-thymidine kinase of *Escherichia coli*. I. Purification and some properties of the enzyme. *Journal of Biological Chemistry* **239**, 269–274.
- OKAZAKI, R. & KORNBERG, A. (1964b). Deoxy-thymidine kinase of *Escherichia coli* II. Kinetics and feedback control. *Journal of Biological Chemistry* **239**, 275–284.
- STOCK, D. A. & GENTRY, G. A. (1971). Thymidine metabolism in *Mycoplasma hominis*. *Journal of General Microbiology* **65**, 105–107.
- TAKEYA, K., SHIMAMOTO, M. & MIZUGUCHI, Y. (1978). Physicochemical and biological properties of mycobacteriocin M12 produced by *Mycobacterium smegmatis* ATCC 25855. *Journal of General Microbiology* **109**, 215–223.
- WACHSMAN, J. T. & MORGAN, D. D. (1971). Deoxy-ribonucleoside kinases of *Bacillus megaterium* KM. *Journal of Bacteriology* **105**, 787–792.
- WAYNE, L. G., DOUBEK, J. R. & RUSSELL, R. L. (1964). Classification and identification of mycobacteria I. Tests employing Tween 80 as substrate. *American Review of Respiratory Disease* **90**, 588–597.
- YAGIL, E. & ROSNER, A. (1970). Effect of adenosine and deoxyadenosine on the incorporation and breakdown of thymidine in *Escherichia coli* K-12. *Journal of Bacteriology* **103**, 417–421.