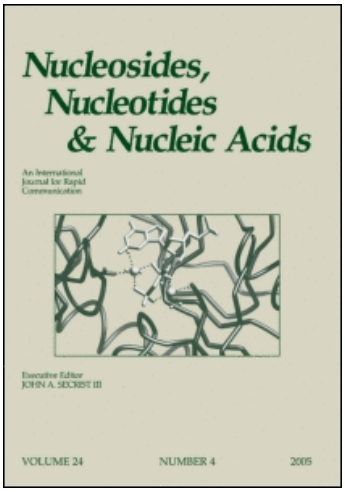


**Nucleosides, Nucleotides and Nucleic Acids**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

# *Pasteurella Multocida* Thymidine Kinase 1 Efficiently Activates Pyrimidine Nucleoside Analogs

A. R. Clausen[a]; S.A. L. Al Meani[a]; J. Piškur[a]
[a] Department of Cell and Organism Biology, Lund University, Sölvegatan, Lund, Sweden

Online publication date: 11 June 2010

**To cite this Article** Clausen, A. R. , Al Meani, S.A. L. and Piškur, J.(2010) '*Pasteurella Multocida* Thymidine Kinase 1 Efficiently Activates Pyrimidine Nucleoside Analogs', Nucleosides, Nucleotides and Nucleic Acids, 29: 4, 359 — 362
**To link to this Article: DOI:** 10.1080/15257771003729716
**URL:** http://dx.doi.org/10.1080/15257771003729716

*Nucleosides, Nucleotides and Nucleic Acids*, 29:359–362, 2010

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257771003729716

Taylor & Francis
Taylor & Francis Group

# *PASTEURELLA MULTOCIDA* THYMIDINE KINASE 1 EFFICIENTLY ACTIVATES PYRIMIDINE NUCLEOSIDE ANALOGS

**A. R. Clausen, S. A. L. Al Meani, and J. Piškur**
*Department of Cell and Organism Biology, Lund University, Sölvegatan 35, SE-22362, Lund, Sweden*

□ *In the* Pasteurella multocida *genome only one putative deoxyribonucleoside kinase encoding gene, for thymidine kinase 1 (PmTK1), was identified. The PmTK1 gene was sub-cloned into* Escherichia coli *KY895 and it sensitized the host towards 2′,2′-difluoro-deoxycytidine (gemcitabine, dFdC), 3′-azido-thymidine (AZT) and 5-fluoro-deoxyuridine (5F-dU). PmTK1 was over-expressed and purified with two different tags. Apparently, deoxyuridine (dU), and not thymidine (dT), is the preferred substrate. We suggest that PmTK1s could be employed as a species-specific activator of uracil-based nucleoside antibiotics.*

**Keywords** Thymidine kinase; nucleosides; *Pasteurella multocida*; antibiotics



## INTRODUCTION

*Pasteurella multocida* is a Gram-negative bacterium, which can cause zoonotic infections in humans. Infections can be treated with beta-lactam antibiotics, fluoroquinolones or tetracycline, however the increased use of these antibiotics has already promoted the appearance of resistance strains. The enzymes involved in the metabolism of nucleic acid precursors could represent putative targets and activators for new antibacterial drugs. Thymidine kinase 1 (TK1) catalyzes the reaction: thymidine (dT) + ATP -> thymidine monophosphate + ADP, and is almost ubiquitous in all bacteria.[1] TK1s could be employed as successful activators of nucleoside analogs.[1,2] In this study, we identified a putative TK1 encoding gene, PmTK1, in the *P. multocida* genome. We describe here that if the PmTK1 gene is expressed in *E. coli*, the host becomes sensitive towards a few pyrimidine-based nucleoside analogs. We show that recombinant PmTK phosphorylates preferably deoxyuridine

Address correspondence to A. R. Clausen and J. Piškur, Department of Cell and Organism Biology, Lund University, Sölvegatan 35, SE-22362, Lund, Sweden. E-mail: Anders.Clausen@cob.lu.se; and Jure.Piskur@cob.lu.se

(dU) over dT, and that the obtained kinetic parameters do not necessarily reflects the nucleoside phosphorylating potential in the living cell.

## MATERIALS AND METHODS

### Cloning and Purification

The PmTK1 encoding gene, with accession number DCQ384602, has previously been cloned into the pGEX-2T expression vector from GE Healthcare (P744).[2] PmTK1 was additionally subcloned into the TOPO pET101 vector (Invitrogen) giving the plasmid P993. P744 was over-expressed and purified without cleaving off the glutathione-S-transferase (GST) tag, as reported elsewhere.[2] P993 was over-expressed, giving a C-terminus His-tagged enzyme, and purified as reported elsewhere.[3] Protein purity was determined with SDS-page and the total protein concentration was determined with the Bradford method using BSA as the standard.



### Minimal Inhibitor Concentration (MIC)

The in vivo tests using a kinase deficient *E. coli* strain, KY895, transformed with P744 on the nucleoside analog containing medium were done as reported previously.[2] The nonlabeled nucleosides were from Sigma-Aldrich (St. Louis, MO, USA).

### Enzyme Assay

Radioactive labeled thymidine was from GE-Healtcare, other labeled substrates were from Moravek, California. The enzyme assays have been described elsewhere.[3]

## RESULTS AND DISCUSSION

*Pasteurella multocida* TK1 is closely related to other TK1s from Gram-negative bacteria, such as *E. coli*.[2] So far, Gram-negative TK1s have only been poorly studied. The PmTK1 gene has been introduced into the TK1-defficient *E. coli* KY895 and the host was screened on a variety of nucleoside analogs. The MIC values were very low on 2′,2′-difluoro-deoxycytidine (gemcitabine, dFdC), 5-fluoro-deoxyuridine (5F-dU) and 3′-azido-thymidine (AZT). The host sensitivity towards AZT was at least 3.000-fold higher than the control, for both dFdC and 5F-dU it was 32-fold higher (Table 1). The observed PmTK1 activation ability was then studied on the recombinant PmTK1 carrying two different tags, a C-terminal linked 6xHis and an N-terminal linked GST. We determined the enzyme activity and kinetic parameters with dT, deoxyuridine (dU), deoxyadenosine (dA), deoxyguanosine (dG), deoxycytidine (dC), dFdC, 5F-dU, and AZT.

**TABLE 1** MIC values for three nucleoside analogs. *E. coli* KY895 was transformed with the pGEX-2T vector, either without any insert or with the PmTK1 gene. Data are adapted from ref.[2] LD100 is defined as the lowest concentration of the tested analog that completely inhibits the bacterial growth

| *E. coli* transformed with | LD100(dFdC)/$\mu$M | LD100 (5F-dU)/$\mu$M | LD100(AZT)/$\mu$M |
|---|---|---|---|
| pGEX-2T only | 31.6 | 0.316 | >100 |
| pGEX-2T-PmTK1 (P744) | 1.0 | <0.01 | 0.0316 |

PmTK1 phosphorylated, in the presence of ATP as the donor, dT, dU, 5F-dU, and AZT, but it did not accept as a substrate dA, dG or dC. Surprisingly, also dFdC was not a substrate for either the 6xHis tagged or the GST-tagged PmTK1. For PmTK-6xHis, the $K_{0.5}$ value for dU was 33 $\mu$M, what is 5-fold lower than with dT. The $V_{max}$ value for dU was 0.703 U/mg and for dT it was 1.5-fold higher, giving the $k_{cat}/K_m$ values which were 3-fold higher for dU comparing with dT (Table 2). For GST-PmTK, $K_{0.5}$ for dU was 82 $\mu$M, what was 9-fold lower than for dT. However, the $V_{max}$ values were 6-fold lower for dU compared to dT, resulting in the similar $k_{cat}/K_m$ values for both substrates. It is evident from these data that the GST-tag and 6xHis tag influenced the kinetic parameters of recombinant PmTK1. For GST-PmTK, the kinetics for dT and AZT exhibited positive cooperativity with a Hill coefficient of 1.6 and 1.8, respectively. In contrast, PmTK-6xHis followed the Michaelis-Menten kinetics with dU and dT. GST-PmTK phosphorylated AZT with $K_{0.5}$ of 430 $\mu$M and $V_{max}$ of 0.814 U/mg. For 5F-dU the $K_m$, $V_{max}$ and Hill coefficient values were of 3157 $\mu$M, 2.619 U/mg and 1.9, respectively. Apparently, both analogs were under in vitro conditions not very good substrates, and dFdC was not a substrate at all. However, under in vivo conditions all three tested analogs were successfully phosphorylated (Table 1).

PmTK1 is so far the only TK1, which prefers dU over dT, but the $K_m$ values are high (Table 2). So far, only one more Gram-negative TK1 (SeTK1), from *Salmonella enterica*, has had their kinetic parameters determined. SeTK1

**TABLE 2** Kinetic data for recombinant PmTK1 tagged with either a C-terminal 6xHis or an N-terminal GST-tag. Both recombinant TK1s had lower $K_m$ values for dU than for dT. Both tags may have impaired the kinetic parameters. U: $\mu$mol min$^{-1}$

| | Substrate | $K_{0.5}$/ $\mu$M | $V_{max}$/ U/mg | Hill coefficient | $k_{cat}$/ $s^{-1}$ | $k_{cat}/K_m$/ $s^{-1}\cdot M^{-1}\cdot 10^3$ |
|---|---|---|---|---|---|---|
| PmTK-6xHis | dU | 33 ± 9 | 0.703 ± 185 | 1 | 0.30 | 9.1 |
| PmTK-6xHis | dT | 164 ± 40 | 1.074 ± 158 | 1 | 0.46 | 2.8 |
| GST-PmTK | dU | 82 ± 22 | 0.179 ± 17 | 1 | 0.14 | 1.7 |
| GST-PmTK | dT | 717 ± 62 | 1.122 ± 1 | 1.8 ± 0.4 | 0.90 | 1.3 |
| GST-PmTK | AZT | 430 ± 28 | 0.814 ± 44 | 1.6 ± 0.2 | 0.66 | 1.5 |
| GST-PmTK | 5F-dU | 2298 ± 508 | 2.619 ± 684 | 1.9 ± 0.1 | 1.12 | 0.5 |

phosphorylates dT with $K_m$ of 34 $\mu$M. Two Gram-positive TK1s, BcTK1 (from *Bacillus cereus*),[2] BaTK1 (from *Bacillus anthracis*),[4] and human TK1[5] exhibit low Km values for dT, 0.5–0.6 $\mu$M, while AZT $K_m$ values are of 0.6–4 $\mu$M. SeTK1 is, like PmTK1, not very efficient with AZT having $K_m$ of 73 $\mu$M. However, PmTK1 and SeTK1 efficiently sensitize *E. coli* KY895 giving MIC values for AZT of only 0.01–0.1 $\mu$M [2]. Apparently, Gram-negative TK1s could behave in a different and much more efficient way under *in vivo* conditions. Therefore, the in vitro kinetic parameters obtained with recombinant Gram-negative TK1s only poorly reflect the capacity of these TK1s in the living bacterial cell. In addition, our studies demonstrate that nucleoside analogs could be used as species-specific antibiotics.

## REFERENCES

1. Sandrini, M.P.B.; Clausen, A.R.; Munch-Petersen, B.; Piškur, J. Thymidine kinase diversity in bacteria. *Nucleosides Nucleotides Nucleic Acids*, **2006**, 25, 1153–1158.
2. Sandrini, M.P.B.; Clausen, A.R.; On, S.L.W.; Aarestrup, F.M.; Munch-Petersen, B.; Piškur, J. Nucleoside analogues are activated by bacterial deoxyribonucleoside kinases in a species-specific manner. *J. Antimicrob. Chemother.*, **2007**, 60(3), 510–520.
3. Knecht, W.; Sandrini, M.P.B.; Johnasson, K.; Eklund, H.; Munch-Petersen, B.; Piškur, J. A few amino acid substitutions can convert deoxyribonucleoside kinase specificity from pyrimidines to purines. *EMBO J.* **2002**, 21, 1873–1880.
4. Carnrot, C.; Vogel, S.R.; Byun, Y.; Wang, L.Y.; Tjarks, W.; Eriksson, S.; Phipps, A.J. Evaluation of Bacillus anthracis thymidine kinase as a potential target for the development of antibacterial nucleoside analogs. *Biol. Chem.* **2006**, 387(12), 1575–1581.
5. Munch-Petersen, B.; Tyrsted, G.; and Cloos, L. Reversible ATP-dependent transition between two forms of human cytosolic thymidine kinase with different enzymatic properties. *J. Biol. Chem.* **1993**, 268(21), 15621–15625.