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Kinetics mechanism and regulation of native human hepatic thymidine phosphorylase

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ABSTRACT

Thymidine phosphorylase (TP; EC 2.4.2.4) catalyzes the reversible phosphorolysis of thymidine, deoxyuridine, and their analogues to their respective nucleobases and 2-deoxy-α-D-ribose-1-phosphate (dRib-1-*P*). TP is a key enzyme in the pyrimidine salvage pathways. Activity of the enzyme is crucial in angiogenesis, cancer chemotherapy, radiotherapy, and tumor imaging, Nevertheless, a complete set of kinetic parameters has never been reported for any human TP. This study describes the kinetic mechanism and regulation of native human hepatic TP. The liver is a main site of pyrimidine metabolism and contains high levels of TP. Initial velocity and product inhibition studies demonstrated that the basic mechanism of this enzyme is a sequential random bi-bi mechanism. Initial velocity studies showed an intersecting pattern, consistent with substrate-enzyme-co-substrate complex formation, and a binding pattern indicating that the binding of the substrate interferes with the binding of the co-substrate and *vice versa*. Estimated kinetic parameters were $K_{Thymidine}$ = 284 ± 55, K_{Pi} = 5.8 ± 1.9, $K_{Thymine}$ = 244 ± 69, and $K_{dRib\text{-}1\text{-}P}$ = 90 ± 33 μM. Thymine was a product activator, but becomes a substrate inhibitor at concentrations eight times higher than its K_m. dRib-1-*P* was a non-competitive product inhibitor of the forward reaction. It bounded better to the Enzyme●P_i complex than the free enzyme, but had better affinity to the free enzyme than the Enzyme●Thymidine complex. In the reverse reaction, dRib-1-*P* enhanced the binding of thymine. The enhancement of the thymine binding along with the fact that dRib-1-*P* was a non-competitive product inhibitor suggests the presence of another binding site for dRib-1-*P* on the enzyme.

1. Introduction

Thymidine phosphorylase (TP; EC 2.4.2.4) is an important enzyme of the pyrimidine salvage pathways. It catalyzes the reversible phosphorolysis of the pyrimidine deoxyribosides; thymidine, deoxyuridine, but not deoxycytidine, and their analogues to their respective nucleobases and 2-deoxy-α-D-ribose-1-phosphate (dRib-1-*P*) as follows:

Pyrimidine deoxyriboside + P_i ⇋ Pyrimidine nucleobase + dRib-1-*P*

TP activity is also an essential step in the regulation of intra- or extracellular thymidine concentration, thymidine homeostasis, and angiogenesis in mammalian cells (Janion and Shugar, 1961; Gallo et al., 1967; Schwartz and Milstone, 1988; Schwartz et al., 1988a; and b; Shaw, 1988; Shaw et al., 1988; Folkman, 1990; Fan et al., 1992; Lees and Fan, 1994; Reynolds et al., 1994; Haraguchi et al., 1994; Moghaddam et al., 1995; Miyadera et al., 1995; Brown and Bicknell, 1998; Uchimiya et al., 2002). The enzyme is identical to the platelet derived-endothelial cell growth factor (PD-ECGF) (Usuki et al., 1994; Furukawa et al., 1992). Mutations in the TP gene are associated with mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), an autosomal recessive human disease exhibiting multiple deletions of skeletal muscle. MNGIE patients accumulate thymidine systemically, which ultimately results in imbalances in the mitochondrial pool of deoxyribonucleoside triphosphates that interferes with mitochondrial DNA replication, and in turn causes mitochondrial dysfunction (Nishino et al., 2000). In addition, TP plays a critical role in cancer chemotherapy, radiotherapy as well as tumor imaging. The expression of the enzyme seems to affect sensitivity of the cell to the pyrimidine analogues, as it activates or deactivates some of most frequently used chemotherapeutic pyrimidine nucleoside analogues (Ensminger et al., 1978; el Kouni et al., 1993; Schuller et al., 2000; Tsukamoto et al.,

Abbreviations: dRib-1-*P*, 2-deoxy-α-D-ribose-1-phosphate; K_{ii}, the inhibition constant computed from the replot of intercepts; K_{is}, the inhibition constant computed from the replot of slopes; TP, thymidine phosphorylase (EC 2.4.2.4)

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2000). Furthermore, overexpression of TP has been reported in many primary and metastatic tumors, relative to the surrounding normal tissue (Fox et al., 1996; Higley et al., 1982; Hotta et al. (2004); Imazano et al., 1997). Therefore, specific inhibitors of TP may be useful as chemotherapeutic agents by enhancing the antineoplastic efficacy of some pyrimidine analogues or prevention of angiogenesis and hence tumor growth and metastasis. The search for TP inhibitors could benefit greatly from kinetic studies of the enzyme. Detailed kinetic studies could reveal a great deal about the structure, and function of TP. Such studies are also essential to fully understand the basic reaction mechanism of the enzyme (*e.g.* ping-pong, sequential, random, ordered, *etc.*), and to illustrate the order of binding of the substrates and release of the products. The order of addition of substrates and the mechanism of action of the enzyme would shed some light on the topology of the active center and whether there is a “cooperative effect" between the substrates or not, *etc.* Such information cannot be visualized by x-ray crystallography of the enzyme, but could be critical for interpreting crystallographic results. Thus, kinetic analysis should be a top priority for structure-based strategy for the design, synthesis and evaluation of novel inhibitors of human TP. Nevertheless, a complete set of kinetic parameters has never been achieved for any human TP.

The present study was performed to determine the kinetic parameters of native human hepatic TP. The liver is a major site of pyrimidine metabolism and contains high levels of TP (Ensminger et al., 1978; Kono et al., 1984; Iltzsch et al., 1985; LaCreta et al., 1989; el Kouni et al., 1993; Boschetti et al., 2014). Furthermore, human hepatic TP is also distinct from the enzymes in extrahepatic tissues (*e.g.* placenta) as well as from the liver of other animals in substrate specificity and other characteristics (el Kouni et al., 1993; Oh and el Kouni, 2018).

2. Materials and methods

2.1. Chemicals

[2-^{14}C]thymidine (56 Ci/mol) and [2-^{14}C]thymine (56 Ci/mol) were from Moravek Biochemicals Inc., Brea, CA; Macherey Nagel Polygram Silica Gel G/UV$_{254}$ thin layer chromatography plates from Fisher cientific, NJ; Bio-Rad protein assay kit, from Bio-Rad Laboratories, Hercules, CA. All other chemicals were obtained from Sigma Chemical Co., St. Louis, MO.

2.2. Source of human hepatic TP

Homogenously purified native human hepatic TP was prepared as previously described (Oh and el Kouni, 2018). Protein concentrations were determined by the method of Bradford (1976), as described by Bio-Rad Laboratories, using bovine γ-globulin as a standard.

2.3. Enzyme assay

TP activity was determined by following the formation of [2-^{14}C] thymine from [2-^{14}C]thymidine or *vice versa*. The standard assay mixture contained 40 mM HEPES (pH 7.5), 1 mM DTT, 1 mM EDTA, and 18 μL of enzyme (13.68 ng protein) in a final volume of 36 μL. The reaction was initiated by addition of the enzyme, incubated for 5–20 min at 37 °C, and terminated by boiling in a water bath for 2 min followed by freezing for at least 20 min. Precipitated proteins were pelleted by centrifugation (30,000 x g), and 10 μL of supernatant fluids were spotted on Silica Gel G/UV$_{254}$ TLC plates. The plates were developed in a mobile phase of chloroform, methanol, and acetic acid mixture (90:5:5, v/v/v) till the mobile phase approached the top of the plates. R_f values were 0.4, and 0.7 for thymidine and thymine, respectively. The amount of radioactivity in the substrate and product were detected and quantified on a percentage basis using a Berthold LB-2821 Automatic TLC Linear Analyzer (Wallac Inc., Gaithersburg, MD). This procedure was used for all TP assays unless otherwise specified. Specific activity is calculated as the μmol/mL product formed, as computed from the net % conversion of radiolabeled substrate(s) to radiolabeled product(s) divided by the minutes of incubation and the mg of enzyme protein used.

2.4. Effect of pH on enzyme activity

The effect of pH on the activity of TP was assessed over a pH range of 4.5 to 11.0 in both forward (thymidine phosphorolysis) and reverse (thymidine synthesis). For the forward reaction, assay mixtures contained 40 mM Tris-Citrate (adjusted to the desired pH value), 1 mM DTT, 1 mM EDTA, 1 mM phosphate, 350 μM [2-^{14}C]thymidine (3.2 Ci/mol), 12 μL of enzyme, in a final volume of 24 μL. For the reverse reaction, 0.5 mM [2-^{14}C]thymine (2.24 Ci/mol) replaced thymidine as a substrate and phosphate was removed from the assay mixture. The reactions were incubated for 15 min at 37°C and terminated and analyzed as described above for the standard TP assay.

2.5. Kinetic studies

Kinetic determinations were run under the standard assay conditions, with adjustments to varying ligand concentrations as to insure strict linearity with incubation time and enzyme concentration. Kinetic parameters were estimated by computer programs employing the general methods of Wilkinson (1961) and Cleland (1967) as previously described (Iltzsch et al., 1985; el Kouni et al., 1988; Naguib et al., 2015). This program is designed for the fit of data by the method of least squares directly to a hyperbola, and it provides various kinetic parameters such as the intercepts and slopes of straight lines from the Lineweaver-Burk plots and K_m values, all with their respective standard errors of estimation. The theoretical considerations of Cleland (1963a, b,c, 1967) concerning multi-reactant enzyme kinetics have been applied to the interpretation of the data, and the nomenclature, such as competitive, uncompetitive, noncompetitive, variable substrate, changing fixed substrate, product inhibition, initial velocity-pattern, K_{ii}, K_{is}, linear inhibition, *etc.*, have been used as defined by Cleland (1963a,b,c, 1967). Inhibition constants were estimated from the replots of the intercepts to give K_{ii}, and from replots of the slopes, to give K_{is}. All kinetic parameter values represent means ± S.E.E. from at least two experiments of three replicas each.

3. Results and discussion

3.1. Optimum pH

Maximal enzymatic activities were estimated in a broad range of pH (4.0–11.0) for both the forward (thymidine phosphorolysis), and the reverse (thymidine synthesis) reactions. For thymidine phosphorolysis, maximal activities ranged broadly from pH 5.0 to 8.5 (Fig. 1A). For thymidine synthesis, maximal enzymatic activity occurred at pH 7.5 (Fig. 1B). Therefore, pH 7.5 was chosen as an optimum pH for the kinetic studies.

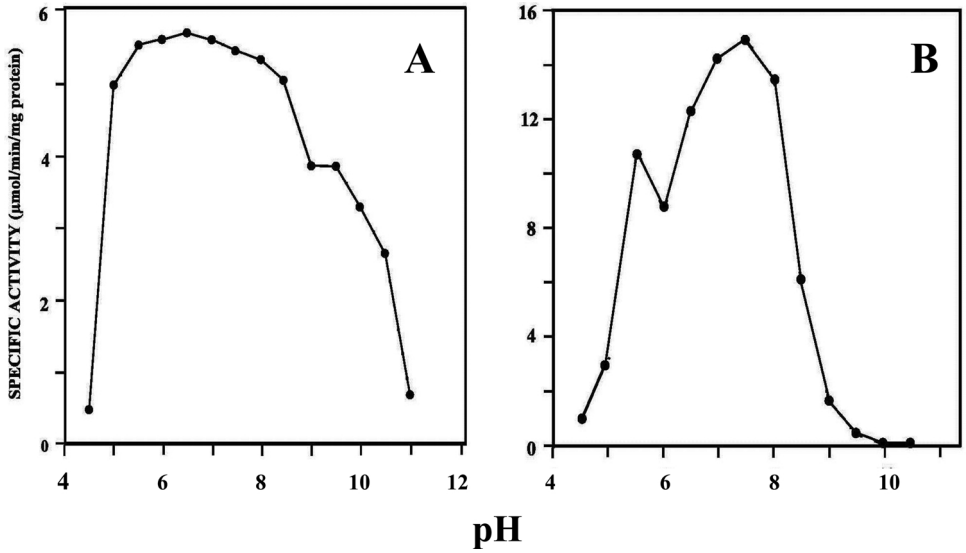

Fig. 1. Effect of pH on the phosphorolysis (**A**) and synthesis (**B**) of thymidine by homogeneously purified hepatic thymidine phosphorylase.

3.2. Initial velocity studies

3.2.1. Thymidine and phosphate as substrates

Initial velocity studies were carried out with varied thymidine (30–720 µM) and increasing fixed concentrations of P_i (6–36 µM) (Fig. 2) as well as with varied P_i (6–36 µM) and increasing fixed of concentrations thymidine (30–720 µM) (Fig. 3). The plot of $1/v$ *vs.*$1/[P_i]$ (Fig. 2), and plot of $1/v$ *vs.* 1/[Thymidine] (Fig. 3) showed an intersection pattern with a common intersection point at coordinates 1/Thymidine = - 0.0012, $1/P_i$ = - 0.059 and $1/v$ = 0.0002. The replots of the slopes and intercepts of the double reciprocal plot were linear (Figs. 2 and 3). The K_m values for thymidine and P_i were calculated from the slope replot of $1/v$-intercepts *vs.* 1/[substrate] and presented in Table 1. $K_{Thymidine}$ (284 ± 55 µM) was higher than that (152 ± 13 µM) reported for the partially purified enzyme (el Kouni et al., 1993). This may suggests the involvement of a biological activator(s) *in vivo*. Thymidine, within the concentrations used (30–720 µM), did not exhibit substrate inhibition (data not shown), contrary to the recombinant TP from human colorectal tumor, where thymidine was reported to be a substrate inhibitor at a concentration above 500 µM (Deves et al., 2014)

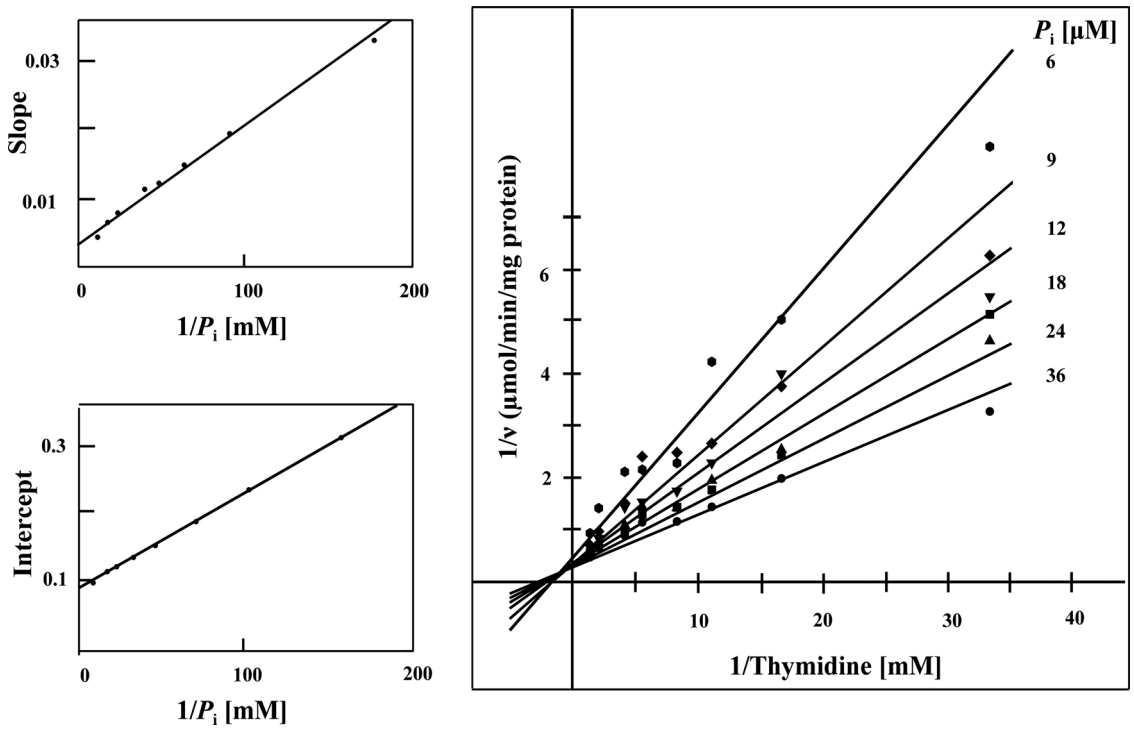

Fig. 2. Effect of phosphate concentration on thymidine phosphorolysis by human hepatic thymidine phosphorylase. Plot of $1/v$ *vs.* 1/[Thymidine] at various fixed concentrations of P_i (6–96 µM), and replots of $1/v$-intercepts and slopes *vs.*$1/[Pi]$. The common intersection point was estimated at coordinates $1/v$-axis = 0.0002, 1/Thymidine = −0.0012.

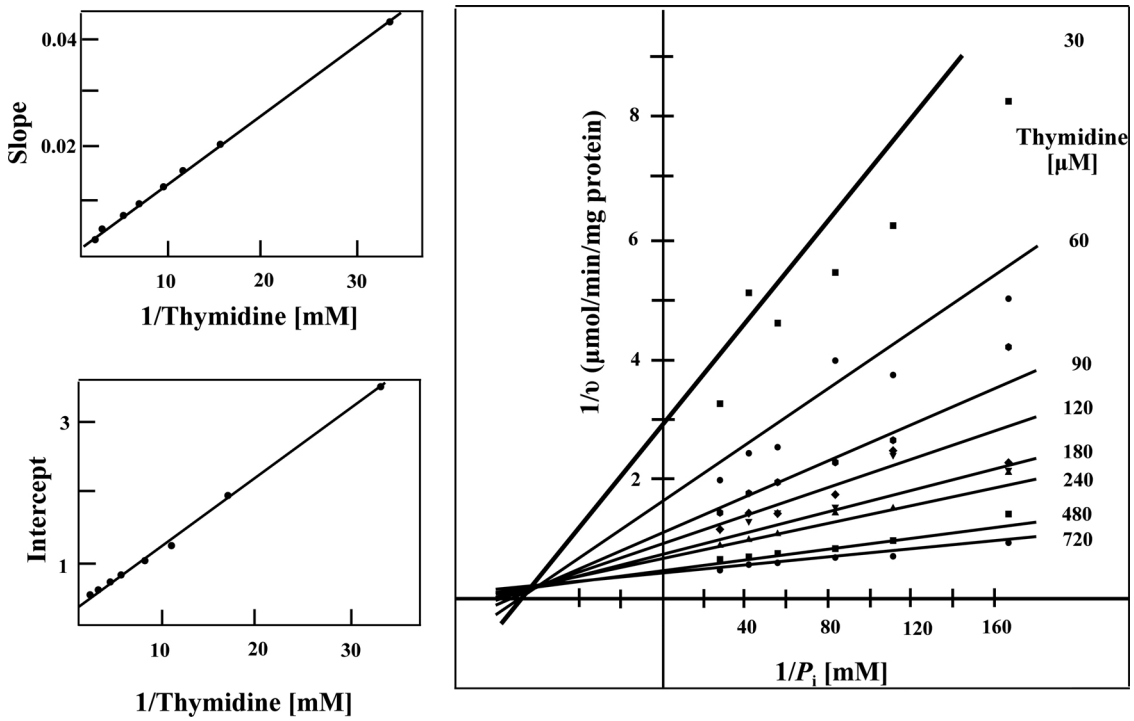

Fig. 3. Effect of thymidine concentration on thymidine phosphorolysis by human hepatic thymidine phosphorylase. Plot of 1/*v vs.* 1/[P_i] at various fixed concentrations of thymidine (30–720 µM), and replots of 1/*v*-intercepts and slopes *vs.*1/[Thymidine]. The common intersection point was estimated at coordinates 1/*v*-axis = 0.0002, 1/P_i = −0.059.

Table 1
Kinetic parameters of native human hepatic thymidine phosphorolysis.

Ligand	K_m [µM]	V_{max} (µmol/min/mg protein)	Efficiency of catalysis (V_{max}/K_m)
Thymidine	284 ± 55[a]	9.9 ± 0.5	0.03
P_i	5.8 ± 1.9	3.5 ± 0.3	0.60
Thymine	244 ± 69	15.3 ± 2.8	0.06
dRib-1-*P*	90 ± 33	15.2 ± 2.3	0.17

[a] Values are means ± standard error of estimation from at least 2 experiments of three replicas each.

3.2.2. *Thymine and dRib-1-P as substrates*

Initial velocity studies were also carried out for the reverse reaction (thymidine synthesis). Figs. 4 and 5 show, the plots of 1/*v vs.* 1/[Thymine] at various fixed concentrations of dRib-1-*P* (30–300 µM), and *vs.* 1/[dRib-1-*P*] at varied fixed thymine concentrations (50–200 µM), respectively. The common intersection points estimated at 1/*v*-axis = -0.0003 and 1/dRib-1-*P* = - 0.0208 (Fig. 4) as well as 1/*v*-axis = -0.0003 and 1/Thymine = - 0.0566 (Fig. 5), were below the x-axis.

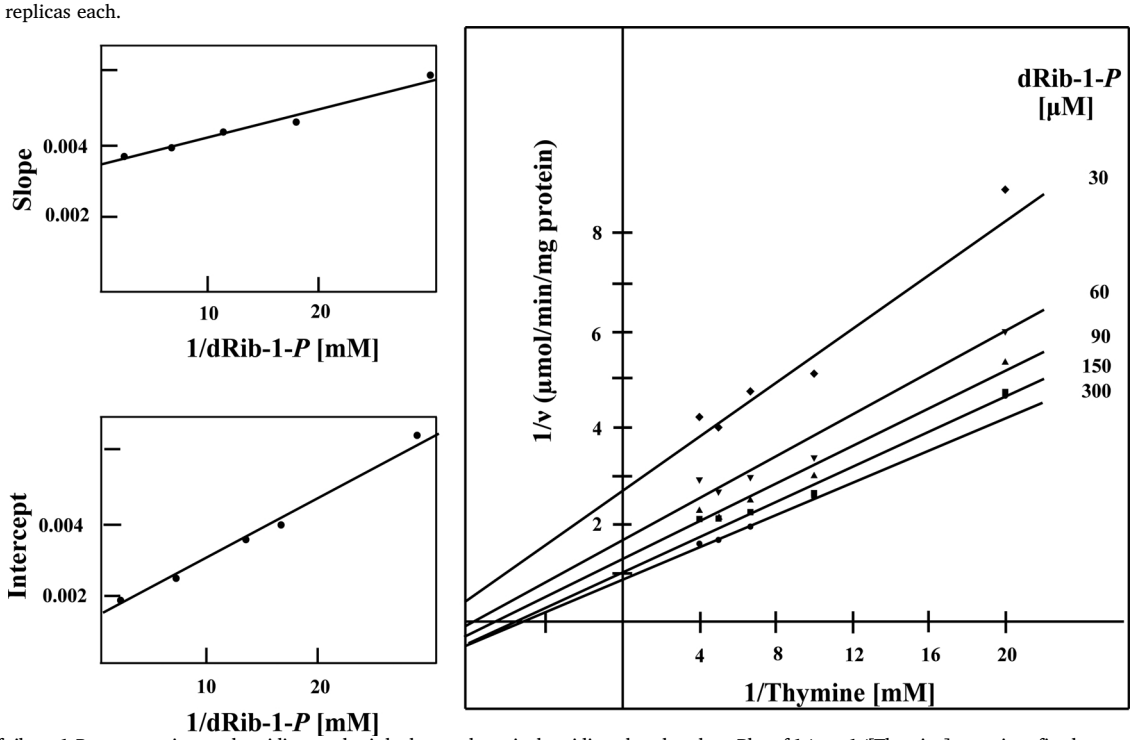

Fig. 4. Effect of ribose-1-*P* concentration on thymidine synthesis by human hepatic thymidine phosphorylase. Plot of 1/*v vs.* 1/[Thymine] at various fixed concentrations of dRib-1-*P* (30–300 µM), and replots of 1/*v*-intercepts and slopes *vs.*1/[dRib-1-*P*]. The common intersection point estimated at coordinates 1/*v*-axis = - 0.0003, 1/dRib-1-*P* = - 0.0208.

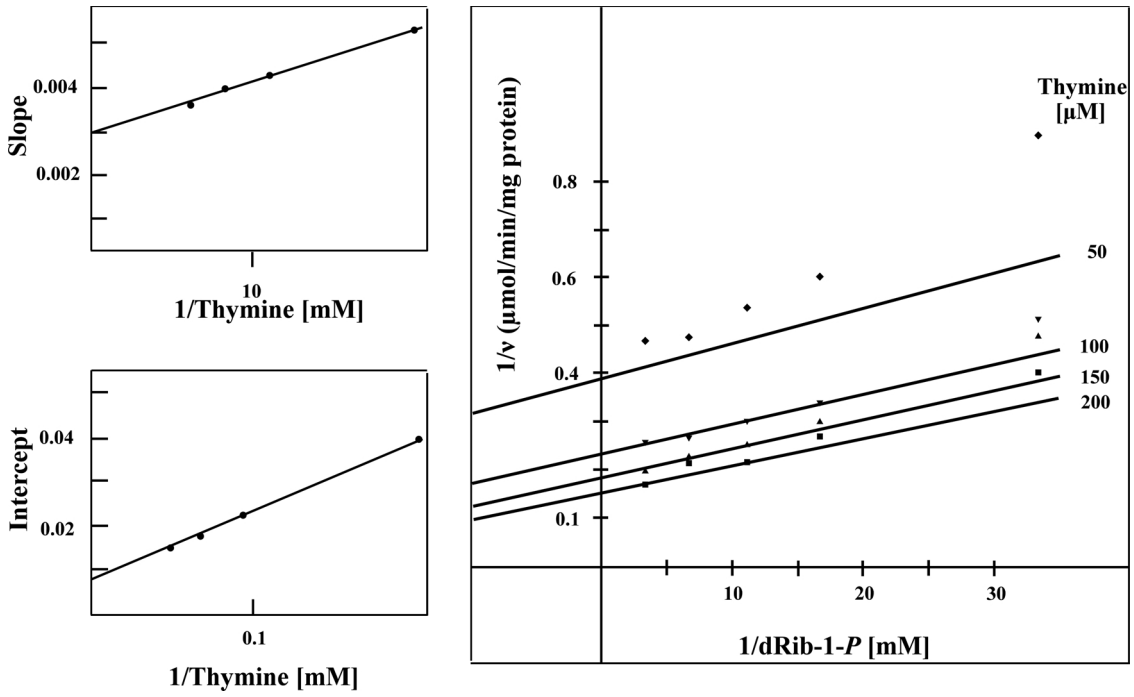

Fig. 5. Effect of thymine concentration on thymidine synthesis by human hepatic thymidine phosphorylase. Plot of $1/v$ *vs.* 1/[dRib-1-*P*] at various fixed concentrations of thymine (50–250 µM), and replots of $1/v$-intercepts and slopes *vs.*1/[Thymidine]. The common intersection point estimated at coordinates $1/v$-axis = - 0.0003, 1/Thymine = - 0.0208.

Under both conditions, forward (Figs. 2 and 3) and reverse (Figs. 4 and 5) reactions, the replots of the slopes and intercepts of the double reciprocal plots were linear. The replots also showed a decrease in both K_{ii} and K_{is}. Consequently, the binding of one substrate seems to increase the binding of the other, *i.e.*, the binding of P_i enhances the affinity of the enzyme for thymidine and *vice versa,* and the binding of dRib-1-*P* enhances the affinity of the enzyme for thymine and *vice versa*. Fig. 6 shows that TP activity increased with the increase of thymine concentrations then gradually decreased at about 1.9 mM (approximately 8 times of its K_m = 245 µM), indicating substrate inhibition by thymine.

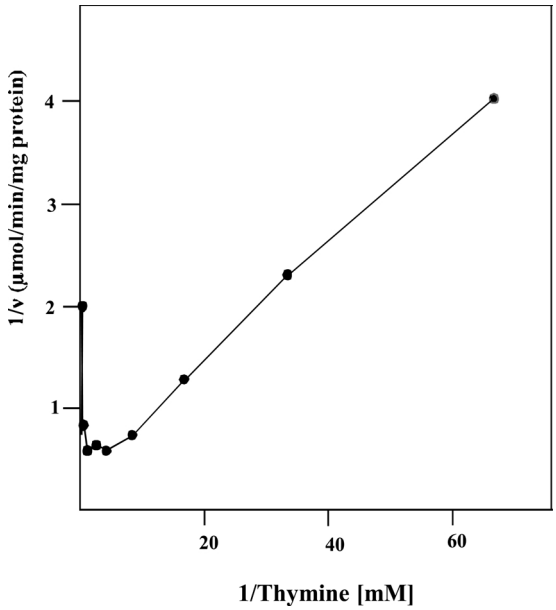

Fig. 6. Substrate inhibition of human hepatic thymidine phosphorylase by thymine (15–3840 µM) at fixed concentration of dRib-1-*P* (1 mM).

The results of the initial velocity studies along with the linearity of the slopes and intercepts of the double replots in the forward and reverse reactions imply that the mechanism of the human hepatic TP is sequential rather than ping-pong. This indicates that both the substrate and cosubstrate bound to the enzyme before any product was released. The results also indicate that thymine inhibits the enzyme by competing with thymidine and P_i for binding to the catalytic site. Thymine was reported to exhibit non-competitive substrate inhibition at high concentration for the mouse liver TP. However, the inhibition of the mouse liver TP, was attributed to the binding of thymine to an effector site inhibiting product formation (Iltzsch et al., 1985).

3.3. Product inhibition studies

Product inhibition studies were conducted to determine whether the addition and release of substrates and products occurred in an ordered or random fashion. Inhibition can be competitive, non-competitive or uncompetitive. This can be determined from the slopes and intercepts of the double reciprocal plots of changing fixed inhibitor concentrations *vs.* varied substrate at a fixed cosubstrate concentrations and *vice versa* (Cleland, 1963a,b). Thymidine and P_i were used as the substrates (substrate and cosubstrate), while either thymine or dRib-1-*P* as used as a product inhibitor and *vice versa*.

3.4. Product inhibition by thymine

Fig. 7 shows the plot of $1/v$ *vs.* P_i and the plot of $1/v$ *vs.* thymidine, when thymine was used as a product inhibitor. The pattern of the double reciprocal plots demonstrated that thymine acted as an activator whether the varied substrate was either P_i (Fig. 7A) or thymidine (Fig. 7B), and the fixed substrate was either P_i or thymidine, respectively. This indicates that thymine is an effector rather than a product inhibitor.

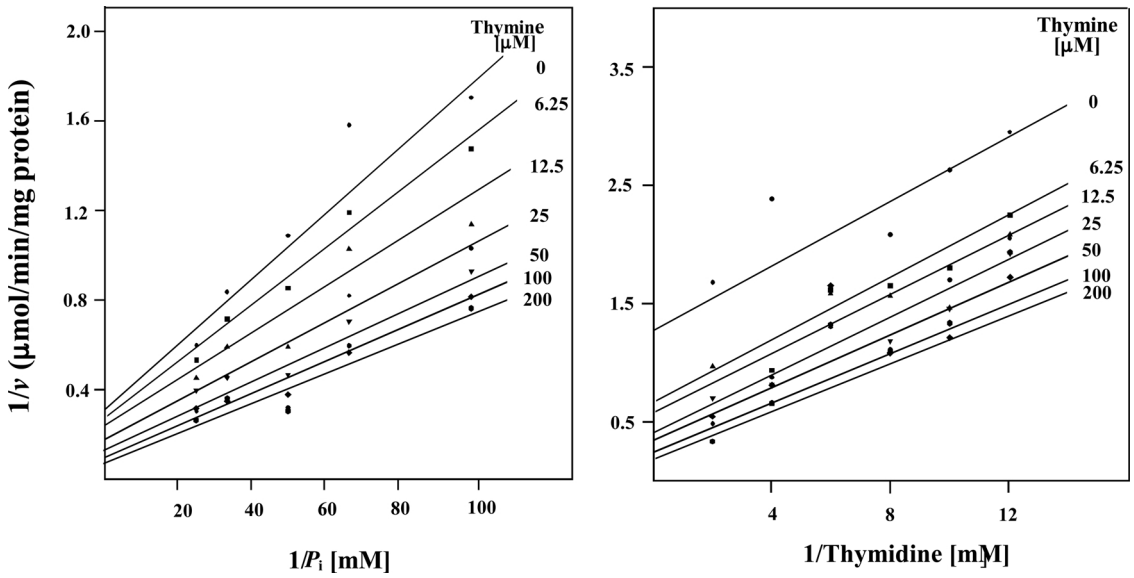

Fig. 7. Product inhibition of human hepatic thymidine phosphorylase by thymine. Effect of various concentrations of thymine (0–200 μM). **A**. Plot of $1/v$ *vs.* $1/[P_i]$ at varied concentrations of P_i (10–40 μM), and fixed K_m concentration of thymidine (284 μM). **B**, Plot of $1/v$ *vs.* 1/[Thymidine] at varied concentrations of thymidine (83–500 μM), and fixed K_m concentration of P_i (5.8 μM).

3.5. Product inhibition by dRib-1-P

Fig. 8 shows the plot of $1/v$ *vs* $[P_i]$ when dRib-1-*P* (0–90 μM) was used as a product inhibitor at various concentrations of P_i (10–40 μM), and a single fixed K_m concentration of thymidine (284 μM). The inhibition constants, K_{ii} (16.3 μM) and K_{is} (82.2 μM) were estimated from the replot of $1/v$-intercepts *vs.* 1/[dRib-1-*P*], and the replot of slope *vs.* 1/[dRib-1-*P*], respectively. Fig. 9, on the other hand, shows the plot of $1/v$ *vs.* 1/[Thymidine] when dRib-1-*P* (0–90 μM) was used as a product inhibitor at various concentrations of thymidine (83–500 μM), and a single fixed K_m concentration of P_i (5.8 μM). The inhibition constants, K_{ii} (21.4 μM) and K_{is} (8.5 μM) were estimated from the replot of $1/v$-intercepts *vs.* 1/[dRib-1-*P*], and the replot of slope *vs.* 1/[dRib-1-*P*], respectively. Under both conditions, whether thymidine (Fig. 9) or P_i (Fig. 8) was the varied substrate and the fixed substrate was P_i or thymidine, respectively, the plot of $1/v$ *vs.* $1/[P_i]$ or *vs.* 1/[Thymidine] yielded a non-competitive inhibition pattern in agreement with a random mechanism. The non-competitive inhibition of the human hepatic TP by dRib-1-*P* differs from that reported for the mouse liver enzyme (Iltzsch et al., 1985) where dRib-1-*P* was a competitive inhibitor.

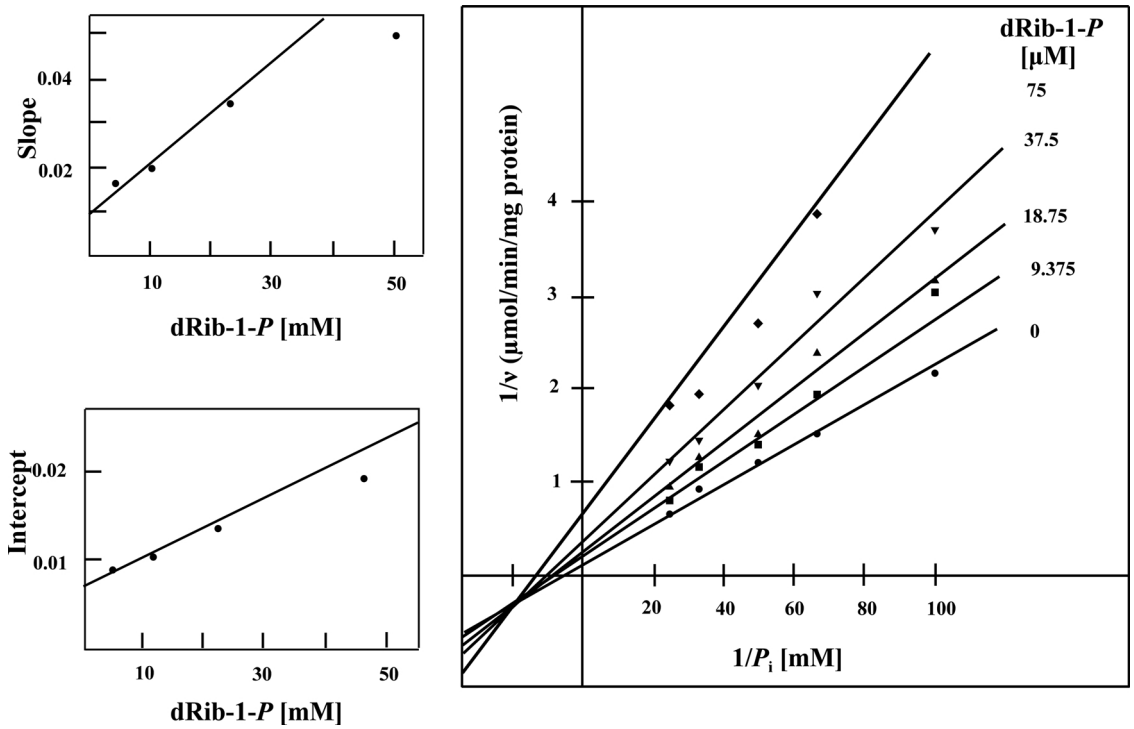

Fig. 8. Product inhibition of human hepatic thymidine phosphorylase by dRib-1-*P* at varied concentrations of P_i (10–40 μM), and fixed K_m concentration of thymidine (284 μM). Plot of $1/v$ *vs.* $1/[P_i]$ at various concentrations of dRib-1-*P* (0–150 μM), and replots of slopes and $1/v$-intercepts *vs.* 1/[dRib-1-*P*]. K_{is} = 82.2 μM, and K_{ii} = 16.3 μM were estimated from the replots.

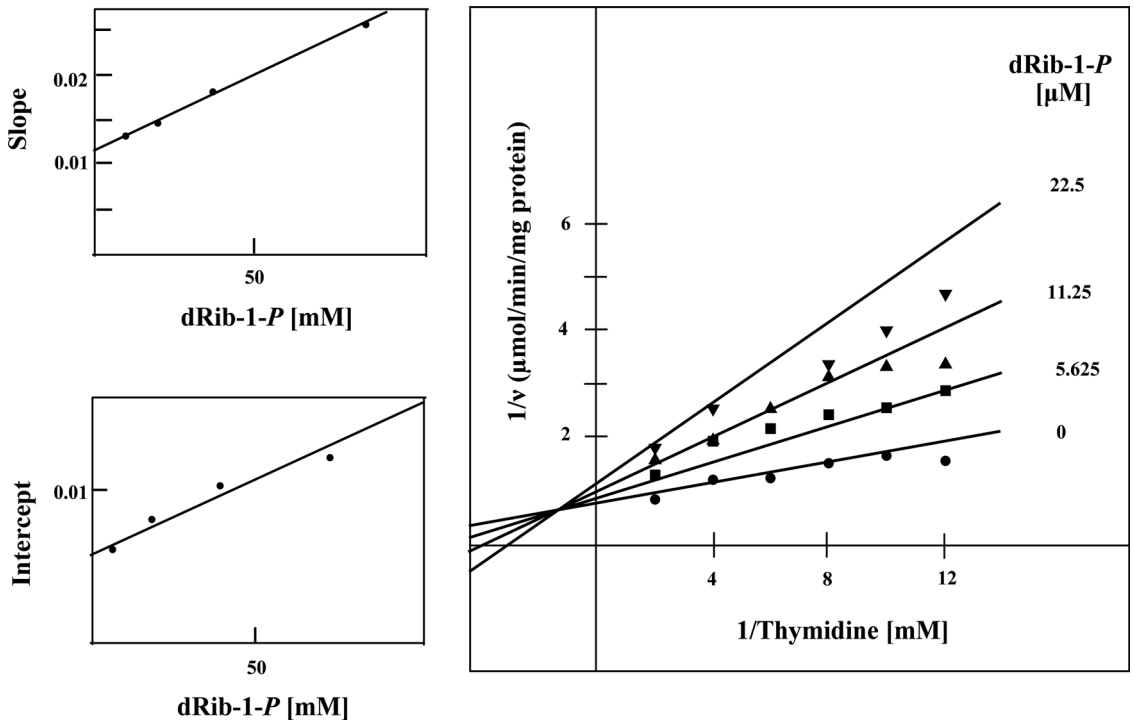

Fig. 9. Product inhibition of human hepatic thymidine phosphorylase by dRib-1-*P* at varied concentrations of thymidine (83–500 μM), and a fixed K_m concentration of P_i (5.8 μM). Plot of 1/*v vs.* 1/[Thymidine] at various concentrations of dRib-1-*P* (0–90 μM), and replots of slopes and 1/*v*-intercepts *vs.* 1/[Thymidine]. K_{is} = 8.5 μM and K_{ii} = 21.4 μM were estimated from the replots.

When dRib-1-*P* was used as a product inhibitor at various concentrations of P_i and a single fixed K_m concentration of thymidine, the position of the intersection point of the double reciprocal plot, was below the x-axis (Fig. 8), and the estimated K_{is} (82.2 μM) was larger than K_{ii} (16.3 μM) suggesting that dRib-1-*P* binds better to the Enzyme●P_i complex than to free enzyme. By contrast, the position of the intersection point of the double reciprocal plot of dRib-1-*P* as a product inhibitor at varied thymidine and fixed P_i concentrations was above the x-axis (Fig. 9), as K_{is} (8.5 μM) was smaller than K_{ii} (21.4 μM), suggesting that dRib-1-*P* has better affinity to free enzyme than to Enzyme●Thymidine complex. Table 2 summarizes the results of the product inhibition studies.

Table 2
The patterns of product inhibitions of native human hepatic thymidine phosphorolysis.

Product Inhibitor	Variable substrate	
	Thymidine P_i fixed at its K_m	P_i Thymidine fixed at its K_m
dRib-1-P	Non-competitive inhibition	Non-competitive inhibition
Thymine	Activation	Activation

4. Conclusions

This is first complete set of kinetic parameters reported for any human TP. In view of the absence of any uncompetitive pattern of the double reciprocal plots, the enzymatic mechanism is consistent with a rapid equilibrium random sequential bi-bi mechanism. dRib-1-*P* was a non-competitive product inhibitor of the forward reaction. It bound better to Enzyme●P_i complex than to free enzyme, but has better affinity to free enzyme than to Enzyme●Thymidine complex. On the other hand, dRib-1-*P* enhanced the binding of thymine in the reverse reaction (Fig. 4). The question then arises as to whether or not there is another binding site for dRib-1-*P* on the enzyme, especially when the product inhibition of varied phosphate by dRib-1-*P* yielded a non-competitive rather than a competitive pattern (Fig. 8). Thymine, on the other hand, was an activator of the forward reaction, but at high concentrations becomes an inhibitor of the reverse reaction (Fig. 6). These results indicate that once thymidine phosphorolysis is initiated, formation of the product thymine will further intensify phosphorolysis to the point of exhaustion. Therefore, it is suggested that the regulation of human hepatic TP would depend on the concentration of thymine rather than dRib-1-*P*. This suggestion is supported by the fact that the intracellular concentrations of dRib-1-*P* is very low as it is promptly metabolized *in vivo* to 2-deoxy-α-D-ribose-5-phosphate, and further to acetaldehyde and glyceraldehyde 3-phosphate by phosphopentomutase (EC 5.4.2.7), and deoxyribose-phosphate aldolase (EC 4.1.2.4), respectively. The overall suggested scheme of reactions between the enzyme and substrates is depicted in Fig. 10.

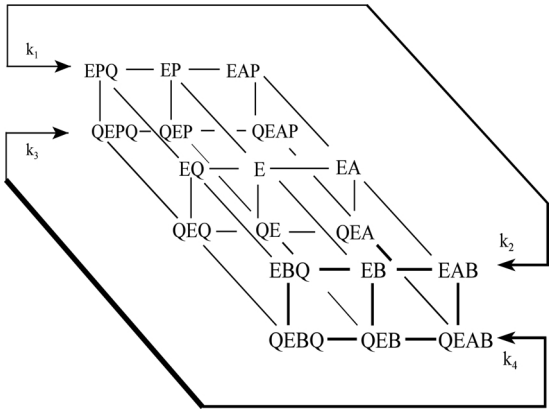

Fig. 10. Scheme of the overall reaction catalyzed by human hepatic thymidine phosphorylase. A, B, P, and Q, represent thymidine; P_i, thymine, and dRib-1-*P*, respectively. The enzyme binds randomly A or B in the forward reaction, and randomly P or Q in the reverse reaction. Since Q inhibits the reverse reaction, Q must bind to an effector site on the enzyme. Because Q also activates phosphorolysis, Q must partake the formation of both EAB and EPQ. When Q occupies its effector site reverse phosphorolysis is inhibited while phosphorolysis is activated. Neither EAP nor EBQ nor their counterparts QEAP or QEBQ are productive enzyme species.

Rapid equilibrium random sequential bi-bi mechanism was also reported for the enzymes from mouse liver (Iltzsch et al., 1985) and human colorectal tumor (Deves et al., 2014). However, the enzymes from the human colorectal tumor and mouse liver differ from the human hepatic TP in some aspects. For example, thymidine was a substrate inhibitor of human colorectal tumor TP (Deves et al., 2014), but not the human hepatic enzyme. Secondly, thymine acted as a product inhibitor of the mouse liver TP (Iltzsch et al., 1985), but was an effector of the human hepatic enzyme. Product inhibition by dRib-1-*P* also differed from the enzyme from mouse liver. dRib-1-*P* was a competitive inhibitor of the mouse liver enzyme (Iltzsch et al., 1985), but a non-competitive inhibitor of the human hepatic enzyme. It is hoped that the present kinetic characterization could shed the light on the roles of TP in pyrimidine metabolism and help in the design of modifiers (*e.g.* inhibitors) for this enzyme.

Acknowledgments

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