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# Kinetic Analysis of Purine Nucleoside Phosphorylase in *Saccharomyces cerevisiae*

**Hye-Seon Choi**

*Department of Microbiology, Ulsan University, Ulsan 680-749, Korea*

**Kinetic parameters of purine nucleoside phosphorylase (PNP) from *Saccharomyces cerevisiae* were measured. The Michaelis constants determined for substrates of the enzyme were $2.0\times10^{-4}$ M for inosine, $2.0\times10^{-3}$ M for deoxyinosine, $2.0\times10^{-5}$ M for guanosine and $2.0\times10^{-5}$ M for deoxyguanosine. According to the ratio of relative $K_{cat}/Km$, substrate specificity of each nucleoside was in the order of guanosine or deoxyguanosine, inosine and deoxyinosine. Cosubstrate, phosphate, revealed downward curvature in Lineweaver-Burk plot at high concentrations, indicating a negative cooperativity between subunits. The inhibition constants for purine analogs were measured to be $6\times10^{-4}$ M for formycin B as the competitive inhibitor of inosine, $9\times10^{-6}$ M for guanine as the competitive inhibitor of guanosine, $2\times10^{-4}$ M for hypoxanthine as the non competitive inhibitor of guanosine and $4.5\times10^{-4}$ M for 6-mercaptopurine as the non competitive inhibitor of guanosine. Alternative substrates, guanosine, deoxyguanosine and adenosine were found to act as competitive inhibitors with Ki values of $2.0\times10^{-5}$ M, $2.6\times10^{-5}$ M and $8.5\times10^{-4}$ M, respectively, when inosine was the variable substrate. Guanosine and deoxyguanosine were also observed as competitive inhibitors with the Ki values of $1.8\times10^{-5}$ M and $3.0\times10^{-5}$ M, respectively, when deoxyinosine was the variable substrate. The results of alternative substrate studies suggested that a single enzyme acted on different nucleosides, inosine, deoxyinosine, adenosine, guanosine and deoxyguanosine.**

**KEY WORDS □ Purine nucleoside phosphorylase, *Saccharomyces cerevisiae***

The relationship between deficiency of purine nucleoside phosphorylase (PNP) and certain immunological disease (22, 26, 28) has made this enzyme a primary target for chemotherapy. Absence of PNP is associated with severe T cell immunodeficiency while B cell immunity remains functional. Therefore, PNP inhibitors might be used to treat T cell leukemia or to counter autoimmune disease without destroying the humoral immunity (4, 8, 27). Inhibitors of PNP might also be useful for enhancing the activities of certain purine nucleoside analogs according to their role in purine metabolism (16, 31, 34, 36). The importance of PNP in immunodevelopment and in the metabolism of purine nucleoside has prompted detailed structural and kinetic studies of the enzyme (3, 5, 6). In addition, the utilization of PNP for the enzymatic production of purine nucleoside analogs, such as adenine arabinoside for an antiviral drug, has encouraged to screen the bacteria which is a potent producer of PNP (20, 21).

The enzyme, PNP, catalyzes the reversible phosphorolysis of inosine, guanosine and their respective deoxynucleosides, using inorganic phosphate as cosubstrate to generate the free base and ribose 1-phosphate or deoxyribose 1-phosphate. At equilibrium, the direction of nucleoside synthesis is favored, but net flux of the reaction in intact cells is in the catabolic direction. PNP also catalyzes the phosphate dependent pentosyl moiety transfer between purine base and nucleoside to produce another purine nucleoside.

PNP has been purified from a variety of sources including human erythrocytes (33, 38), placenta (7), granulocyte (37), bovine brain (18), rabbit brain (17), rabbit liver (19), chicken liver (25), developing embryos of *Hyalomma dromdarii* (14), *Escherichia coli* (15), *Bacillus subtilis* (11) and *Proteus vulgaris* (35). Major physical and kinetic differences in the enzymes from the various sources has been reported (1, 32) and compared to get the information of detailed structural charateristics and reaction mechanism.

In a previous paper (2), PNP from *Saccharomyces cerevisiae* has been partially purified and characterized. This study presented kinetic parameters of nucleoside substrates, inosine, deoxyinosine, guanosine, deoxyguanosine and adenosine and demonstrated the effect of alternative

substrates for a detailed studies of the enzyme. In addition, the phenomenon of negative cooperativity for inorganic phosphate was observed. The inhibition constants of purine analogs for PNP were estimated from replots of double reciprocal plots.

## MATERIALS AND METHODS

**Enzyme Purification**

Baker's Yeast commercially available from Sigma Chemical Co. was used and all purification procedures were followed as described in the previous paper (2).

**Determinations of Kinetic Parameters**

The phosphorolysis of inosine was measured by a spectrophotometric assay coupled to xanthine oxidase (13). The assay was based on the measurement of the increase in absorbance at 293 nm due to the formation of uric acid ($\Delta E = 12.5 \times 10^3\ M^{-1}\ cm^{-1}$ at 293 nm). Spectrophotometer (Kontron, UVKON 860) equipped with RS 232C-interface was used. The reaction mixture contained, in a final volume of 1 m*l*, 50 mM Tris-Cl, pH 7.3, 100 mM potassium phosphate, 0.02 unit of xanthine oxidase and various concentrations of inosine. All reaction mixture except PNP were preincubated at 30°C for about 3 min to remove any trace of hypoxanthine or xanthine as a contaminant in the inosine. The reaction was started by the addition of PNP and continued for about 3 min to get the linear region. When the concentrations of phosphate were variable, the reaction mixture was the same as above except 1 mM of inosine. The phosphorolysis of guanosine was monitored directly at 257 nm with $\Delta E = -5 \times 10^3\ M^{-1}\ cm^{-1}$. The reaction mixture contained 50 mM Tris-Cl, pH 7.3, 100 mM potassium phosphate and various concentrations of guanosine in a 1 m*l* cuvette. The reaction mixture was preincubated for 3 min and the addition of the enzyme initiated the reaction. The reaction was carried out at 30°C and continued for about 2 min. The values of Km and Vm were calculated from Lineweaver-Burk plot. The Ki values for all the inhibitors were obtained in the replot of inhibitor concentrations versus slopes from Lineweaver-Burk plot.

**Data Processing**

Points on the kinetic curves represented average of two or three determinations, but, in the calculations each determination was treated individually. Reciprocal of velocities were plotted graphically versus reciprocal of substrate concentrations. Curves (Fig. 3-11) were fitted to equation

$$1/v = Km/Vm \cdot 1/S + 1/Vm$$

The binding curves for phosphate were evidently not linear, not obeying the above equation. In Fig. 1A, the value of low Km was obtained from extrapolation of 6 points of low concentrations of phosphate and that of high Km was from 6 high concentrations as shown in inset of figure. These curves were fitted to the experimental points according to the Hill equation.

$$v = \frac{V \cdot (S)^{n_H}}{(S)^{n_H} + K}$$

where $n_H$ denoted the Hill coefficient, V the maximum binding and v the amount of ligand bound at a certain concentration of ligand. The Hill coefficient was calculated from the slope of Fig. 1B over a wide range of phosphate concentrations.

The Ki values of inhibitors (Fig 3-11) were calculated from the replots of slopes versus the concentrations of inhibitors shown as insets of figures.

**Determination of Equilibrium Constant**

The reaction mixture contained 50 mM Tris-Cl, pH 7.3, 0.1 mM guanosine and different concentrations (5, 10, 50 mM) of phosphate in a final volume of 1 m*l*. Immediately after adding an appropriate amount of PNP, the absorption spectrum was taken from 220 to 300 nm as a 0 hr sample. The reaction was carried out at 30°C and the absorption spectra at 290 and 350 min were overlapped. At equilibration, the concentration of guanosine and guanine were measured and the equilibium constant was calculated. The conversion of guanosine to guanine gives the spectral change at 257 nm ($\Delta E = -5 \times 10^3\ M^{-1}\ cm^{-1}$)

## RESULTS

**Kinetic Parameters for PNP**

The effects of inosine, deoxyinosine, guanosine and deoxyguanosine concentration on PNP activity were determined in 100 mM potassium phosphate at 50 mM Tris, pH 7.3. The Michaelis constants determined in Lineweaver-Burk plot were $2.0 \times 10^{-4}$ M, $2.0 \times 10^{-3}$ M, $2.0 \times 10^{-5}$ M and $2.0 \times 10^{-5}$ M for inosine, deoxyinosine, guanosine, and deoxyguanosine, respectively.

The interesting feature of PNP reaction was shown when the effect of phosphate on enzyme activity was measured as the concentration of inosine was fixed at 1 mM. Fig. 1A illustrated the biphasic nature of a double-reciprocal plot with phosphate as the variable substrate, characterizing by a downward curvature of the line. Two values of Km for phosphate were extrapolated to be $1.1 \times 10^{-3}$ M and $3.6 \times 10^{-3}$ M for the concentration of phosphate of 0.5~5.0 mM and 5.0~50 mM, respectively. The deviation from linearity of the plot was apparant at phosphate

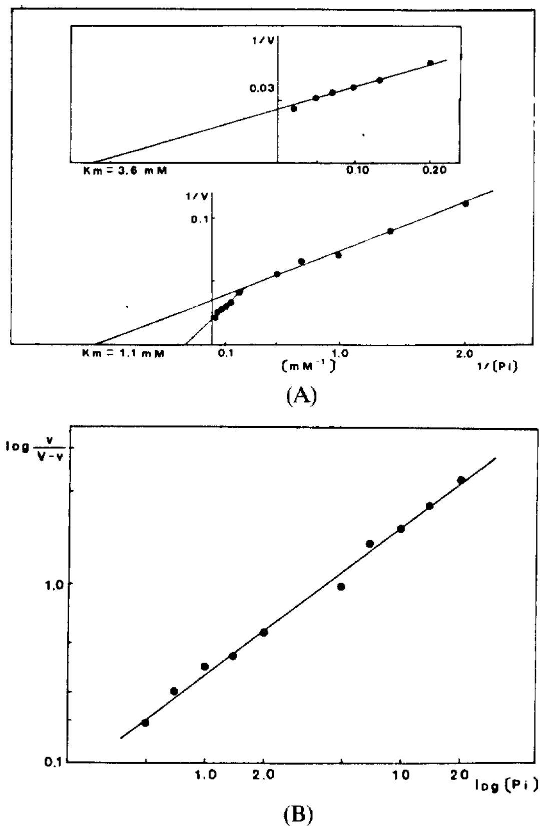



**Fig. 1.** *(A) Lineweaver-Burk plot of purine nucleoside phosphorylase (PNP) from Saccharomyces cerevisiae with phosphate as a variable substrate.*
The reaction mixture contained in a volume of 1 m*l*: 50 mM Tris-Cl, pH 7.2, 1 mM inosine, 0.02 units of xanthine oxidase, variable concentrations (0.5~50 mM) of phosphate and PNP. The plot for high concentrations (5~50 mM) of phsphate were enlarged in the inset. The values of Km of phosphate were $1.1\times10^{-3}$ M and $3.6\times10^{-3}$ M for the concentrations of phosphate of 0.5~5 mM and 5~50 mM; respectively.
*(B) Hill plot of the same data according to the equation, log v/V−v=$n_H$ log (Pi)−log K.*
The value of $n_H$ was calculated to be 0.70.

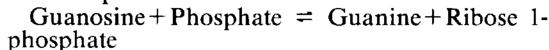
concentrations greater than 5.0 mM. This phenomenon seemed to be caused by a possible cooperativity between the subunits. A Hill plot of the phosphate was shown in Fig. 1B. The calculated data from Fig. 1B gave a straight line with a Hill coefficient of about 0.70, indicating a negative cooperativity in binding of phosphate to the enzyme.

**Table 1.** *Kinetic parameters for substrates of PNP from Saccharomyces cerevisiae*

| Substrate | Km (mM) | Relative $K_{cat}$ | Relative $K_{cat}$/Km |
|---|---|---|---|
| Inosine | 0.2 | 1 | 5.0 |
| Deoxyinosine | 2.0 | 1.4 | 0.7 |
| Guanosine | 0.02 | 0.54 | 27 |
| Deoxyguanosine | 0.02 | 0.54 | 27 |

Table 1 showed kinetic parameters for substrates for PNP. The value of relative $K_{cat}$ for deoxyinosine was 1.4 folds greater than that for inosine. Thus, at high concentrations deoxyinosine appeared to be a better substrate than inosine, if two reactions occurred separately. However, each substrate cannot be considered in isolation from the other under physiological conditions. When the substrates are mixed together, the ratio of rates of the competing reactions could be determined by the ratio of relative $K_{cat}$/Km. The rate of phosphorylation of inosine was 7 times faster than that of deoxyinosine for an equimolar mixture of inosine and deoxyinosine at any concentration, showing that inosine is the more specific substrate. The values of relative $K_{cat}$ for guanosine and deoxyguanosine were estimated to be 0.54 fold lower than that for inosine. However, since the ratios of relative $K_{cat}$/Km for guanosine and deoxyguanosine were calculated to be 5.5 folds greater than that for inosine, guanosine and deoxyguanosine were considered to be better substrates.

The equilibrium constant of the reaction:



Guanosine + Phosphate ⇌ Guanine + Ribose 1-phosphate

was determined from the ratio of the equilibrium concentrations and calculated to be 0.048 (Fig. 2). This reaction was performed with different concentrations of phosphate and the value was averaged.

The affinities of purine analogs for *Saccharomyces cerevisiae* PNP were determined. Formycin B, inosine analog, did not act as a substrate. However, when inosine was a variable substrate at a constant level of phosphate, formycin B acted as a competitive inhibitor with a Ki value of $6\times10^{-4}$ M (Fig. 3). Guanine, hypoxanthine and 6-mercaptopurine were tested as inhibitors when guanosine was a variable substrate at a fixed level of phosphate. Guanine was observed as a competitive inhibitor with a Ki value of $9\times10^{-6}$ M (Fig. 4). Hypoxanthine and 6-mercaptopurine showed noncompetitive patterns with Ki values of $2\times10^{-4}$ M (Fig. 5) and $4.5\times10^{-4}$ M (Fig. 6), respectively.

**Effects of alternative substrates**

It was demonstrated in the previous paper (2)

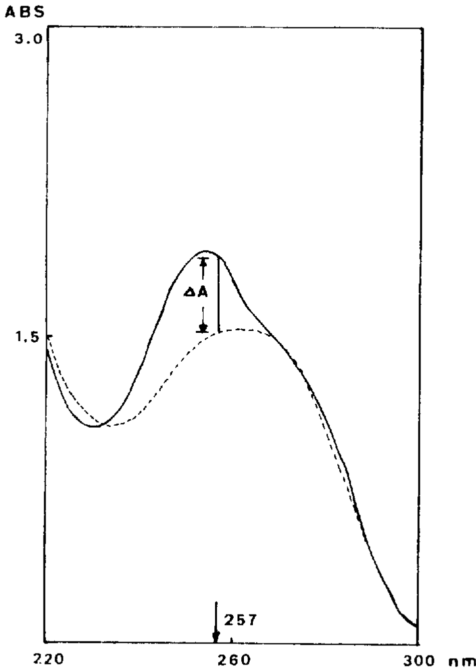



**Fig. 2.** *Absorption spectrum of the conversion of guanosine to guanine by purine nucloside phosphorylase (PNP).*
The reaction mixture contained 50 mM Tris, pH 7.3 , 96 $\mu$M guanosine (E=$13.7\times10^3$ $M^{-1}$ $cm^{-1}$ at 252.5 nm) and 5 mM of phosphate in a final volume of 1 m*l*. Immediatly after adding an appropriate amount of PNP, the absorption spectrum was taken from 220 to 300 nm as 0 hr sample (__). The reaction mixture was incubated at 30°C and the spectrums at 290 and 350 min (---) were overlapped. The phosphorolysis of guanosine was measured at 257 nm with $-\Delta E=5\times10^3$ $M^{-1}cm^{-1}$ as described in Materials and Methods. At equilibrium the concentration of guanine was determined to be 72.5 $\mu$M. The equilibrium constant at 5 mM phosphate was calculated to be 0.047. Those at 10 mM and 50 mM phosphate were 0.050 and 0.046, respectively. The value of equilibrium constant for PNP was averaged.

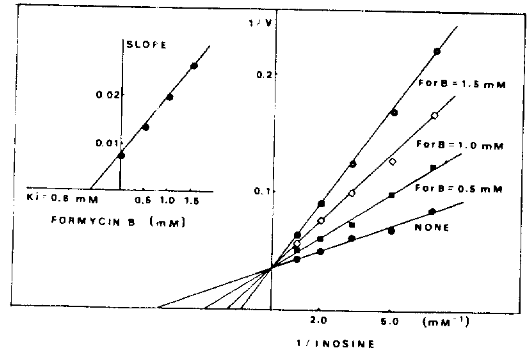



**Fig. 3.** *Inhibition by substrate analog, formycin B, when inosine was the variable substrate.*
Plot of reciprocal of the initial velocity with respect to reciprocal of inosine concentration. The reaction mixture was prepared and the reaction was carried out as described in Matrials and Methods. Inosine and formycin B were added in the concentration indicated. The inhibition constant, Ki of formycin B as the competitive inhibitor of inosine, was estimated to be $6\times10^{-4}$ M by the replot shown in the inset.

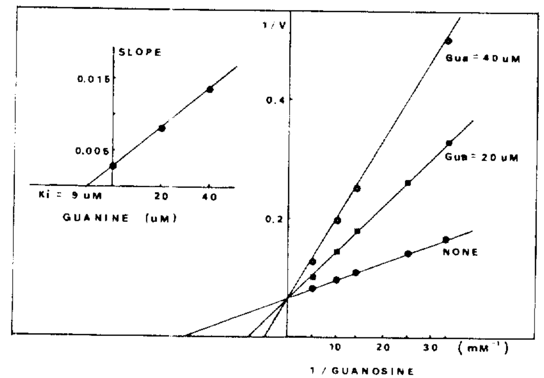



**Fig. 4.** *Inhibition by the purine base, guanine, when guanosine was the variable substrate.*
Plot of reciprocal of initial velocity with respect to reciprocal of guanosine concentration. The inhibition constant, Ki of guanine as the competitive inhibitor of guanosine, was estimated to be $9\times10^{-6}$ M by the replot shown in the inset.

that PNP from *S. cerevisiae* was capable of catalyzing the phosphorolysis of inosine, deoxyinosine, guanosine and deoxyguanosine. Adenosine seemed to be phosphorolyzed with a relatively slow rate. The compounds of inosine, deoxyinosine, adenosine, guanosine and deoxyguanosine could react as alternative competing substrates.

In order to study the effect of alternative substrates, the rate of product was determined when an alternative substrate was tested as an inhibitor. When inosine was the variable substrate at a constant level of phosphate, guanosine and deoxyguanosine were observed as competitive inhibitors. As shown in Fig. 7 and 8, the rate of hypoxanthine was measured by the coupled assay. The inhibition constants, Ki, of guanosine and deoxyguanosine were calculated to be $2.0\times10^{-5}$

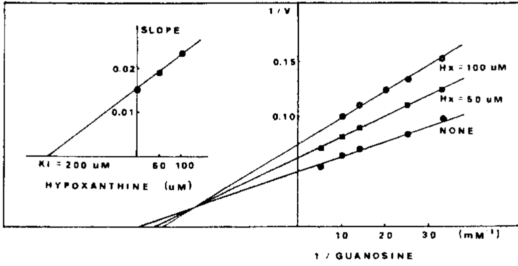



**Fig. 5.** *Inhibition by the purine base, hypoxanthine, when guanosine was the variable substrate.* Plot of reciprocal of initial velocity with respect to reciprocal of guanosine concentration. The inhibition constant, Ki of hypoxanthine as the non competitive inhibitor of guanosine, was estimated to be $2\times10^{-4}$ M by the replot shown in the inset.

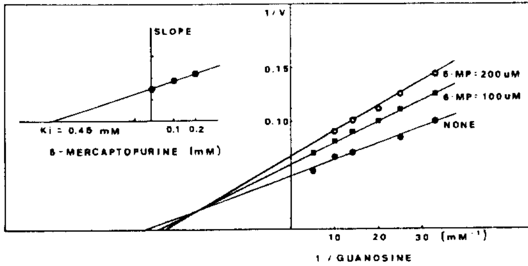



**Fig. 6.** *Inhibition by the purine base, 6-mercaptopurine, when guanosine was the variable substrate.*
Plot of reciprocal of initial velocity with respect to reciprocal of guanosine concentration. The inhibition constant, Ki of 6-mercatopurine as the non competitive inhibitor of guanosine, was estimated to be $4.5\times10^{-4}$ M by the replot shown in the inset.

M and $2.6\times10^{-5}$ M, respectively, from the replot of slope versus the concentration of each competing substrate. As shown in Fig. 9, adenosine was also observed as a competitive inhibitor with a Ki value of $8.5\times10^{-4}$ M. The inhibition constant of adenosine was much higher than those of guanosine and deoxyguanosine. Fig. 10 and 11 showed that the ribo and deoxyribonucleoside of guanine were found to act as competitive inhibitors when deoxyinosine was variable substrate at a constant level of phosphate. The inhibition constants of guanosine and deoxyguanosine, estimated by the replots, were 1.8 $\times10^{-5}$ M and $3.0\times10^{-5}$ M, respectively. These results were consistent with the hypothesis that the enzyme has only one catalytic site for four different nucleosides, inosine, deoxyinosine, guanosine and deoxyguanosine.

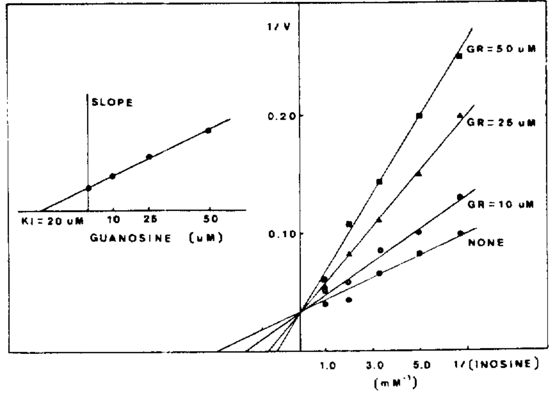



**Fig. 7.** *Inhibition by the alternative substrate, guanosine, when inosine was the variable substrate.* Plot of reciprocal of the initial velocity with respect to reciprocal of inosine concentration. The inhibition constant, Ki of guanosine as the competitive inhibitor of inosine, was estimated to be $2.0\times10^{-5}$ M by the replot shown in the inset.

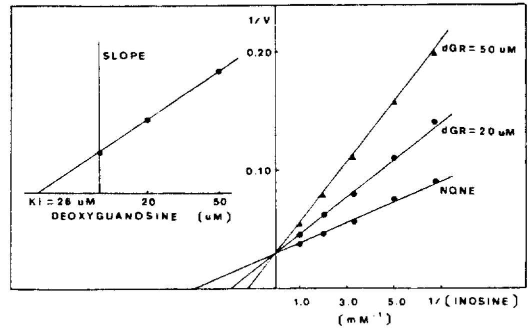



**Fig. 8.** *Inhibition by the alternative substrate, deoxyguanosine, when inosine was the variable substrate.*
Plot of reciprocal of the initial velocity with respect to reciprocal of inosine concentration. The inhibition constant, Ki of deoxyguanosine as the competitive inhibitor of inosine, was estimated to be $2.6\times10^{-5}$ M by the replot shown in the inset.

## DISCUSSION

Purification of PNP from *S. cerevisiae* has been previously reported and the enzyme has been characterized (2). In the present studies, these characterization have been extended by kinetic analysis.

The affinities of various nucleosides to the

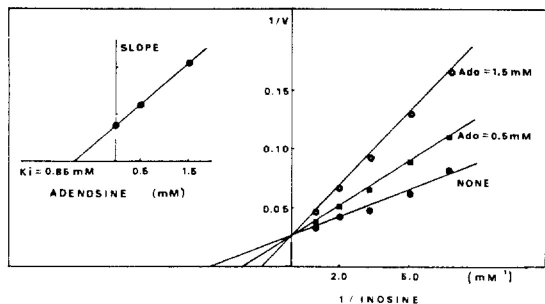



**Fig. 9.** *Inhibition by the alternative substrate, adenosine, when inosine was the variable substrate.*
Plot of reciprocal of the initial velocity with respect to reciprocal of inosine concentration. The inhibition constant, Ki of adenosine as the competitive inhibitor of inosine, was estimated to be $8.5\times10^{-4}$ M by the replot shown in the inset.

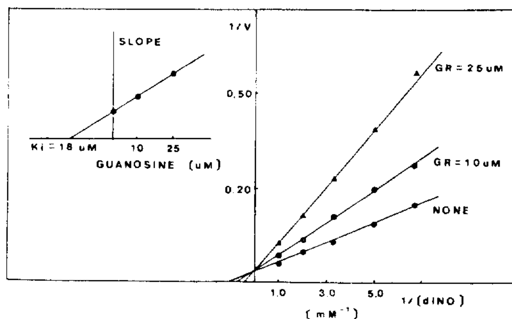



**Fig. 10.** *Inhibition by the alternative substrate, guanosine, when deoxyinosine was the variable substrate.*
Plot of reciprocal of the initial velocity with respect to reciprocal of deoxyinosine concentration. The inhibition constant, Ki of guanosine as the competitive inhibitor of deoxyinosine, was estimated to be $1.8\times10^{-5}$ M by the replot shown in the inset.

enzyme were determined. Practically identical Km values were found for guanosine and deoxyguanosine. The Km value of guanosine was 10 fold lower than that of inosine. When the hydroxyl group of inosine was replaced by a hydrogen atom, as in deoxyinosine, an increase in Km values was found. The affinities of alternative substrates was in the order of guanosine or deoxyguanosine, inosine and deoxyinosine.

The observed substrate activation at high concentration of phosphate suggested that PNP can be a multivalent enzyme with cooperative interaction between active sites. Another ex-

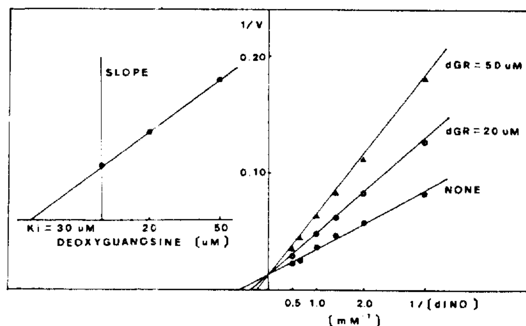



**Fig. 11.** *Inhibition by the alternative substrate, deoxyguanosine, when deoxyinosine was the variable substrate.*
Plot of reciprocal of the initial velocity with respect to reciprocal of deoxyinosine concentration. The inhibition constant, Ki of deoxyguanosine as the competitive inhibitor of deoxyinosine, was estimated to be $3.0\times10^{-5}$ M by the replot shown in the inset.

planation for non-linear Lineweaver-Burk plots could be that PNP from *S. cerevisiae* was a mixture of two or more enzymes with widly different affinities for phosphate. However, the latter seemed to be ruled out. In this case, it was to be expected that the Lineweaver Burk plots for another substrate, inosine, would be non linear. The double reciprocal plots for inosine was observed to be linear up to 1.5 mM. The similar substrate activation at high concentration of phosphate has been reported with bovine thyroid PNP (24), bovine liver PNP (10), bovine spleen PNP (29), human erythrocytic PNP (32), rabbit erythrocytic PNP (30) and PNP from *Escherichia coli* and *Salmonella typhimurium* (12). It was demonstrated that negative cooperativity at high inosine concentration was seen with human erythrocytic PNP (32), bovine spleen PNP (29) and chicken liver enzyme (25).

It was reported by Jensen (11) that PNP from *Bacillus subtilis* had the apparent Km values of 0.2 and 3.9 mM for inosine and phosphate, respectively. The apparent Km values for inosine and deoxyinosine were 50 and 47 $\mu$M and for phosphate, 0.37 mM for PNP from *Salmonella typhimurium* (9). When the catalytic properties were measured in human erythrocytic PNP, the Km values of inosine, deoxyinosine, guanosine and phosphate were demonstrated to be 48, 66, 47 and 32 $\mu$M, respectively (33).

PNP from *Saccharomyces cerevisiae* was capable of catalyzing the phosphorolysis of inosine, deoxyinosine, guanosine and deoxyguanosine. From the value of relative $K_{cat}$, deoxyinosine was better substrate than inosine. Inosine was faster than

guanosine or deoxuguanosine for the relative velocity of the enzyme. However, each substrate cannot be considered separately under physiological conditions. When the substrates existed simultaneously, the ratio of rates for each nucleoside could be estimated by the value of relative $K_{cat}/Km$. Guanosine or deoxyguanosine was better substrate than inosine for PNP from *S. cerevisiae*, since the value of relative $K_{cat}/Km$ of guanosine or deoxyguanosine was 5.5 times higher than that of inosine.

The equilibrium constant for phosphorolysis was calculated to be 0.048. Equilibrium favors the reversible reaction, nucleoside synthesis. The value was approximately equal to equilibrium constants from mammalian tissues and microorganisms (25).

Various purine analogs were tested as an inhibitor for PNP from *S. cerevisiae*. Inosine analog, formycin B acted as a competitive inhibitor, but had no substrate activity, suggesting that formycin B could bind the enzyme without converting product. Guanine was found to have better affinity for PNP than hypoxanthine. The value of Ki for guanine ($9\times10^{-6}$ M) was one order lower than that for hypoxanthine ($2\times10^{-4}$ M). That phenomenon was true when they acted as a substrate. The Michaelis constants of guanosine and inosine were $2\times10^{-5}$ M and $2\times10^{-4}$ M, respectively.

The effects of alternative substrates were observed when these compounds were treated as inhibitors. Each different nucleoside, guanosine, deoxyguanosine and adenosine, was found to act as a competitive inhibitor when inosine or deoxyinosine was the variable substrate at a fixed level of phosphate. These results suggested that a single enzyme acted on all nucleosides tested, inosine, deoxyinosine, guanosine, deoxyguanosine and adenosine. It could not be possible that there are more than one enzyme, each highly specific for each differnt nucleoside. None of the purified mammalian PNP enzymes has not been reported to use the nucleobase, adenine or its analog as a substrate. It was demonstrated that PNP from *B. subtilis* consisted of two enzymes, inosineguanosine phosphorylase and adenosine phosphorylase (11). Adenosine phosphorylase was found to be different from inosine-guanosine phosphorylase in chemical and physical properties in *E. coli* (15) and *Acholeplasma laidlawii* (23), PNP from *S. cerevisiae* might have more than one catalytic center with competing alternative substrates. Further studies with competing alternative substrates and more purified enzyme could give detailed information on PNP from *S. cerevisiae*.

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**초 록: *Saccharomyces cerevisiae*에서 얻은 Purine Nucleoside Phosphorylase의 반응 속도론적 분석**

**최혜선** (울산대학교 미생물학과)

*Saccharomyces cervisiae*에서 얻어진 purine nucleoside phosphorylase(PNP)의 반응속도론적 상수들이 측정되어졌다. Nucleoside 기질들의 Michaelis 상수는 inosine이 $2.0\times10^{-4}$M, deoxyinosine이 $2.0\times10^{-3}$ M, guanosine이 $2.0\times10^{-5}$ M이고 deoxyguanosine이 $2.0\times10^{-5}$ M이었다. 상대적 $K_{cat}/Km$ 값에서 nucleoside의 기질 특이성은 guanosine이나 deoxyguanosine, inosine, 그리고 deoxyinosine의 순이었다. 또 다른 기질인 phosphate는 Lineweaver-Burk plot에서 높은 농도지역에서 밑으로 구부러진 곡선을 보여주며 하위단위체간의 음성적 협동성을 시사하고 있다. Purine 유사체들의 inhibition constant는 formycin B가 inosine의 경쟁적 억제자로서 $6.0\times10^{-4}$ M, guanine이 guanosine의 경쟁적 억제자로서 $9.0\times10^{-6}$ M, hypoxanthine이 guanosine의 비경쟁적 억제자로서 $2.0\times10^{-4}$ M, 6-mercaptopurine이 guanosine의 비경쟁적 억제자로서 $4.5\times10^{-4}$ M이었다. Inosine이 변화하는 기질일 때 guanosine, deoxyguanosine과 adenosine은 경쟁적 억제자로 작용하고 각각 $2.0\times10^{-5}$ M, $2.6\times10^{-5}$ M, $8.5\times10^{-4}$ M의 Ki 값을 가지고 deoxyinosine이 변화하는 기질일 때도 guanosine과 deoxyguanosine은 경쟁적 억제자로서 $1.8\times10^{-5}$ M과 $3.0\times10^{-5}$ M의 Ki 값이 얻어졌다. 대체 기질인 nucleoside들을 억제자로 조사한 연구는 *S. cerevisiae*의 PNP는 각기 다른 nucleoside인 inosine, deoxyinosine, adenosine, guanosine과 deoxyguanosine를 기질로 쓸 수 있는 한개의 효소로 이루어져 있음을 시사했다.