The Journal of Microbiology, March 1997, p.15-20

Vol. 35, No. 1

Catalytic Mechanism and Inhibition Studies of Purine Nucleoside Phosphorylase (PNP) in *Micrococcus luteus*

Hye-Seon Choi

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

(Received December 13, 1996 / Accepted January 24, 1997)

Kinetic studies were done to elucidate the reaction mechanism of purine nucleoside phosphorylase (PNP) in *Micrococcus luteus*. PNP catalyzes the reversible phosphorolysis of ribonucleosides to their respective base. The effect of alternative competing substrates suggested that a single enzyme was involved in binding to the active site for all purine nucleosides, inosine, deoxyinosine, guanosine, deoxyguanosine, adenosine and deoxyadenosine. Affinity studies showed that pentose moiety reduced the binding capacity and methylation of ring N-1 of inosine and guanosine had little effect on binding to bacterial enzyme, whereas these compounds did not bind to the mammalian enzymes. The initial velocity and product inhibition studies demonstrated that the predominant mechanism of reaction was an ordered bi, bi reaction. The nucleoside bound to the enzyme first, followed by phosphate. Ribose 1-phosphate was the first product to leave, followed by base.

Key words: Purine nucleoside phosphorylase, reaction mechanism, *Micrococcus luteus*

Purine nucleoside phosphorylase (PNP) catalyzes the reversible phosphorolysis of inosine, guanosine, and their respective deoxynucleosides. The enzyme needs inorganic phosphate as cosubstrate to produce the free base and ribose 1-phosphate or deoxyribose 1-phosphate. At equilibrium, nucleoside synthetic direction is favored, but net flux 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 nucleoside. The association between deficiency of PNP and certain immunological disease (19, 21, 23) has drawn attention to the enzyme as a promising chemotherapeutic target. Deficiency of PNP enzyme causes severe T cell immunodeficiency while B cell immunity is intact. Therefore, PNP inhibitor can be used to treat T cell leukemia or lymphoma or as a selective immunosuppressant without destroying humoral immunity (9, 22). PNP inhibitors could be useful for improving the potency of clinically used nucleoside analogs cleaved by erythrocytic PNP. Consequently, considerable efforts has been directed towards discovering inhibitors of the enzyme (6, 8, 15, 25, 27, 29). In addition, the use of bacterial PNP in biosynthesis of clinically useful adenosine analogs has encouraged screening of the bacteria that produce substantial amounts of the enzyme (18). The enzyme has been isolated from both eukaryotic and prokaryotic organisms (2, 10, 11, 12, 16, 17, 20, 26, 28) and investigated for detailed structural studies (5, 7) and kinetic mechanisms (1, 3, 13, 26). *Micrococcus luteus* PNP has been purified in this laboratory and characterization of its physical and kinetic properties has been described previously (4). The catalytic mechanism has been studied from the initial velocity and product inhibition studies. The inhibition constant of purine nucleoside analogs was determined in order to get some information about the active site of PNP.

Materials and Methods

Chemicals

Inosine, guanosine, guanine, hypoxanthine, xanthine oxidase from butter milk, Tris, DEAE-Sephadex A-50, Sephadex G-100, Sephadex G-150, streptomycin sulfate, and ammonium sulfate were purchased from Sigma Chemical Co. PM-10 membrane filter was supplied from Amicon. All the other chemicals used in the study were of the highest quality.

Enzyme purification

Micrococcus luteus KCTC 1071 was purchased and used as enzyme source. All purification procedures were followed as described in the previous paper (4). Aliquots of Purified PNP fraction were

lyophilized and stored frozen at -70°C.

Determination of kinetic parameters

The phosphorolysis of inosine was measured by a spectrophotometric assay coupled to xanthine oxidase (14). The assay was based on the measurement of the increase in absorbance at 293 nm due to the formation of uric acid ($\Delta E=12.5\times10^{3}\ M^{-1}\ cm^{-1}$ at 293 nm). A temperature controlled spectrophotometer (Kontron, UVKON 860) equipped with RS 232C-interface was used. The reaction mixture contained in a final volume of 1 ml, 50 mM Tris, pH 7.0, various concentrations of inosine, 20 mM of potassium phosphate, 0.02 unit of xanthine oxidase, and an appropriate amount of enzyme, PNP. The entire reaction mixture except PNP was preincubated for 3 min to remove any trace amount of hypoxanthine or xanthine as a contaminant in the commercially available inosine preparation. The reaction was initiated by the addition of the enzyme, and continued for about 2 min to get a linear response. When the concentrations of phosphate were varieov, the reaction mixture was the same as above except for containing 10 mM of inosine. The Ki values for all the inhibitors were obtained in the replot of inhibitor concentrations versus slopes from Lineweaver-Burk plot or Dixon plot. Points on the kinetic curves represented average of two or three determinations, but each determination was calculated individually. Reciprocals of velocities were plotted graphically versus reciprocals of substrate concentrations. Curves were fitted to equation. 1/v= Km/Vm · 1/s+1/Vm

Results

Effect of alternative substrates

In the previous paper (4), substrate specificity of PNP from *Micrococcus luteus* was determined for various nucleosides. The order of substrate specificity was inosine, guanosine, deoxyguanosine, and deoxyinosine. The nucleosides inosine, deoxyinosine, guanosine, and deoxyguanosine reacted as alternative competing substrate. The effect of alternative substrates on the reaction velocity was investigated, 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 found to be competitive inhibitors. The Ki values of guanosine and deoxyguanosine were calculated from replot to be 6.0×10^{-4} M and 1.2×10^{-3} M, respectively (Table 1). Adenosine and deoxyadenosine also showed competitive inhibition patterns. The inhibition constants of adenosine and deoxyadenosine were estimated from replot of slope versus the concentration of inhibitors to be 1.3×10^{-3} M and 1.1×10^{-3} M, respectively. Guanosine and deoxyguanosine were also competitive inhibitors with Ki values of 3.0×10^{-4} M and 4.0×10^{-4} M, respectively when deoxyinosine was a variable substrate. These results suggested that the enzyme has one catalytic site for different purine nucleosides. Inosine, deoxyinosine, guanosine, deoxyguanosine, adenosine, and deoxyadenosine appeared to be held in a common binding site. In order to study the effect of alternative substrates, the combined rate of formation of product from both alternative substrates was measured. When the concentrations of inosine, deoxyinosine or inosine with constant level of deoxyinosine was changed, the reaction velocity was determined. If the enzyme has only one catalytic site for both inosine and deoxyinosine, the combined rates of the reaction are calculated by

Table 1. Inhibition constants and inhibition patterns of alternative substrates with PNP from *Micrococcus luteus*

Inhibitor	Variable Substrate	Ki (M)	Type of Inhibition
Guanosine	Inosine	6.0×10^{-4}	Competitive
Deoxyguanosine	〃	1.2×10^{-3}	〃
Adenosine	〃	1.3×10^{-3}	〃
Deoxyadenosine	〃	1.1×10^{-3}	〃
Guanosine	Deoxyinosine	3.0×10^{-4}	〃
Deoxyguanosine	〃	4.0×10^{-4}	〃

$$v=\frac{\frac{VS}{K}\frac{V'S'}{K'}}{\frac{1+S}{K}+\frac{S'}{K'}} \quad (1)$$

where V, K and S are maximal velocity, Michaelis constant, and concentration of inosine, respectively, V', K' and S' are the signs for deoxyinosine and v is the combined rate. However, if each nucleoside needs two different catalytic sites, that is, there are two enzymes, the combined velocities are given by

$$v=\frac{VS}{K+S}+\text{velocity with 10 mM of deoxyinosine} \quad (2)$$

As shown in Fig. 1, Line y4 and Line y5 were drawn by equations 1 and 2, respectively, from the calculation of experimental points for inosine (y1) and deoxyinosine (y2). Line y4 agrees with the experimentally observed combined velocities (y3) better than Line y5, excluding that there are two different enzymes, one specific for inosine and the other specific for deoxyinosine.

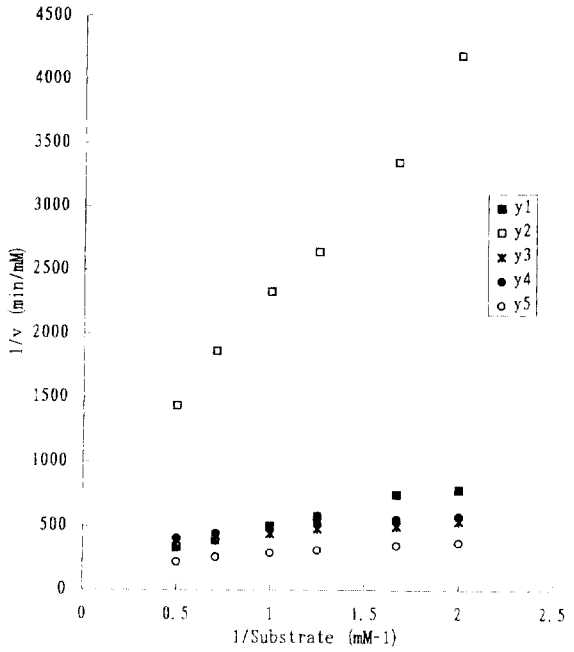

Fig. 1. Double reciprocal plot of purine nucleoside phosphorylase with inosine, deoxyinosine or both as a variable substrate. The reaction mixture contained, in a final volume of 1 ml, 50 mM Tris, pH 7.0, 20 mM of potassium phosphate, 0.02 units of xanthine oxidase, PNP and various concentrations of nucleoside substrate as indicated; y1, rate with inosine; y2, rate with deoxyinosine; y3, rate with inosine in the constant amount (10 mM) of deoxyinosine; y4, rate calculated on the basis of equation 1; y5, rate calculated on the basis of equation 2.

The effects of inhibitors

The affinities of various purine nucloside analogs and bases for PNP from *Micrococcus luteus were* determined. The Ki values and the inhibition patterns of each compound are presented in Table 2. Of the compounds surveyed, inhibition constants of uridine and thymidine were not determined due to an interference with high absorbance. On the contrary, the affinities of the corresponding bases, uracil, and thymine increased enough to measure the Ki values. In a similar manner, 6-mercaptopurine riboside and 6-mercaptopurine, adenosine and adenine, or guanosine and guanine showed 5-10 fold differences in Ki values.

Table 2. Inhibition constants and inhibition patterns of various compounds of PNP from *Micrococcus luteus*

Compounds	Ki (uM)	Inhibition Pattern
Guanosine	600	competitive
Deoxyguanosine	1200	competitive
Adenosine	1300	competitive
Formycin B	60	competitive
Guanine	60	competitive
6-Mercaptopurine	20	competitive
6-Mercaptopurine riboside	100	noncompetitive
8-Aminoguanosine	40	noncompetitive
1-Methylinosine	1500	noncompetitive
7-Methylinosine	400	noncompetitive
1-Methylguanosine	330	noncompetitive
7-Methylguanosine	950	noncompetitive
Adenine	250	noncompetitive
Uracil	1000	noncompetitive
Thymine	1000	noncompetitive

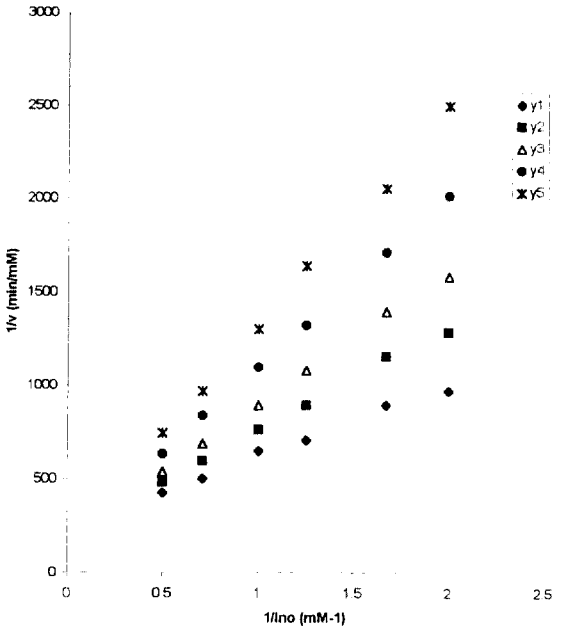

Fig. 2. The initial velocity study with inosine as a variable substrate at a fixed concentration of phosphate.The reaction mixture contained, in a final volume of 1 ml, 50 mM Tris, pH 7.0, 0.02 units of xanthine oxidase, PNP, various concentrations of inosine and indicated concentrations of potassium phosphate; y1, 1.4 mM; y2, 2 mM; y3, 3 mM: y4, 5 mM; y5, 10 mM.

Initial velocities

Measurements of initial velocities gave a pattern of lines in the double reciprocal plot which distinguished sequential from ping-pong mechanisms, when one substrate was changed while the other was fixed at different constant concentrations. Fig. 2 shows the initial velocity pattern for PNP from *Micrococcus luteus* when the inosine concentration is varied at different fixing concentrations of phosphate. The double reciprocal plots for initial velocity intersected on the x coordinate with linear replots of slopes and intercepts. A similar pattern of reciprocal plots was also observed when phosphate was varied at constant levels of inosine (Results not shown). These results indicated a sequential mechanism for phosphorolytic reaction of the enzyme. Kinetic constants, estimated from the replot, are listed in Table 3.

Table 3. Kinetic constants for the phosphorolytic reaction of PNP from *Micrococcus luteus*

Kinetic Constant	value
K_a	1.4×10^{-3} M
K_b	2.0×10^{-3} M
K_{ia}	3.5×10^{-3} M

The constants K_a and K_b were limiting Michaelis constants for inosine and phosphate, respectively. These values were calculated from replot of double reciprocal plots.

Table 4. Product inhibition patterns for the reactions of PNP from *Micrococcus luteus*

Inhibitor	Variable Substrate	Fixed Substrate	Type of Inhibition
Guanine	Inosine	Pi (1.5 mM)	competitive
Guanine	Inosine	Pi (20 mM)	competitive
Guanine	Pi	Inosine (1.5 mM)	noncompetitive
Guanine	Pi	Inosine (10 mM)	noncompetitive
R-1-P	Inosine	Pi (1.5 mM)	noncompetitive
R-1-P	Inosine	Pi (20 mM)	uncompetitive
R-1-P	Pi	Inosine (1.5 mM)	noncompetitive
R-1-P	Pi	Inosine (1.5 mM)	noncompetitive

Product inhibition

Sequential mechanisms could be random, ordered bi, bi or Theorell-Chance, a limiting case of the ordered mechanism. Product inhibition studies have been recognized as diagnostic of the distinguishing characteristics of the above mechanisms. When phophate was the variable substrate at a saturating concentration of inosine, the inhibition of one product, guanine disappeared. An unsaturating concentration of inosine (1.5 mM), guanine was observed to inhibit noncompetitively. In a similar manner, inosine was varied at fixed concentrations of phosphate (1.5 mM and 20 mM) and guanine was a competitive inhibitor of phosphorolysis. This suggested that guanine and inosine compete for the same site of the enzyme. Double reciprocal plots of initial velocities with varing concentrations of inosine at an nonsaturating concentration of phosphate (1.5 mM) using the product ribose 1-phosphate as an inhibitor, showed that the inhibition was non-competitive. At saturating concentrations of phosphate (20 mM), the inhibition pattern was changed to be noncompetitive. The inhibitory effect of ribose 1-phosphate was tested when phosphate was the variable substrate at a fixed concentration of inosine. Both concentrations of 1.5 mM and 10 mM inosine gave similar non-competitive inhibition patterns. Product inhibition studies for the phosphorolytic reaction of PNP from *Micrococcus luteus* are summarized in Table 4. The results were consistent with an ordered bi, bi mechanism, in which the nucleoside is the first substrate to add to the enzyme and the purine base is the last product to dissociate from the enzyme.

Discussions

Alternative substrate and inhibition studies have been employed in developing the conformational and configurational constraints of the nucleoside in the active site. These studies have also provided an insight into the possible enzymatic mechanism of the substitution reaction.

The effect of alternative competing substrates were observed under two conditions. In one case, the combined effect of inosine and deoxyinosine was observed. The experimental line was compared with calculated lines based on equations. The possibility that there are two enzymes, each specific for inosine or deoxyinosine was excluded. The assumption that there is a single enzyme active for inosine and deoxyinosine seemed to be more valid. In the other case, the formation of a product derived from only one substrate was measured. Guanosine, deoxyguanosine, adenosine or deoxyadenosine was noted to inhibit the enzyme competitively when inosine or deoxyinosine was a variable substrate at a fixed concentration of phosphate. These results were consistent with the above finding that a single enzyme was involved in phosphorolysis of all nucleosides, inosine, deoxyinosine, guanosine, deoxyguanosine, adenosine, and deoxyadenosine.

The affinities of various nucleosides and bases for PNP from *Micrococcus luteus* were measured. The inhibition constants of bases were much lower than those of corresponding nucleosides (Ki of adenosine and adenine; 1300 and 250 uM; Ki of 6-mercaptopurine riboside and 6-mercaptopurine; 100 and 20 uM; Ki of guanosine and guanine; 600 and 60 uM). The pentose moiety of the compound did not help binding to the enzyme. The 2'-hydroxyl group moderately affected the affinity for the enzyme (Ki of adenosine and deoxyadenosine: 1300 and 1100 uM; Km of inosine and deoxyinosine; 1500 and 3000 uM; Km of guanosine and deoxyguanosine: 500 and 500 uM (4)). Formycin B which can effectively bind but is not a substrate, acted as a competitive inhibitor. The dramatic increase in affinity for the enzyme on replacement of N-9 by C-9 was noted (Ki of formycin B: 66 uM; Km of inosine: 1500 uM). The substitution of S for C-6 also increase the affinity, but that of NH_2 for the same site did not change the affinity (Ki of 6-mercatopurine riboside: 100 uM; Ki of adenosine: 1300 uM). Addition of an electron donating amine to C-8 showed better affinity than guanosine did

(Ki of guanosine and 8-aminoguanosine; 600 and 40 uM). Methylation of ring N-7 did not abolish the binding capacity. In general, alterations of the purine imidazole ring generated good affinity. Methylation of the ring N-1 of inosine or guanosine had little effect on affinity (Km of inosine: 1500 uM; Ki of 1-methylinosine: 1500 uM; Ki of guanosine and 1-methylguanosine: 600 and 330 uM). This is consistent with the report that 1-methylinosine and 1-methylguanosine were neither substrates nor inhibitors of calf spleen PNP, but both compounds were almost as good as substrates as the parent nucleosides for *Escherichia coli* PNP (1).

When inosine was the variable substrate with phosphate as a fixed substrate, the Lineweaver-Burk plots showed a crossing pattern in which the slope and intercept changed. Similar patterns were observed when phosphate was a variable substrate with inosine as a fixed substrate. It was concluded from these findings that the predominant mechanism of PNP from *Micrococcus luteus* was not a ping-pong mechanism. Product inhibition studies were performed to distinguish the sequence of substrates and products. The inhibition by the products guanine or ribose 1-phophate was examined when inosine or phosphate was the variable substrate below and above saturating concentrations of the other substrate. The observed competitive inhibition by guanine, when either inosine or phosphate concentration was varied, indicated that three ligands, inosine, phosphate, and guanine can bind to the free enzyme. The noncompetitive inhibition with ribose 1-phosphate could be explained by assumption that the pentose moiety did not bind to the free enzyme form. The initial velocity and product inhibition studies were consistent with the mechanism being an ordered bi, bi reaction. It appeared that the nucleoside bound to the enzyme first, followed by phosphate. An isomerization between complexes of enzyme and substrates and that of enzyme and products occurred and ribose 1-phosphate left the enzyme first with purine base being the last product to leave the enzyme. This mechanism was consistent with that for human erythrocytic PNP (26).

In the case of PNP from *Saccharomyces cerevisiae*, the binding sequence of substrates was reverse, while the reaction mechanism agreed with ordered bi, bi kinetics (3).

Substrate binding experiments with labeled substrates will give more information to elucidate the mechanism of action of PNP from *Micrococcus luteus*.

Acknowledgments

This work was supported by research grants from Ulsan University, 1996.

References

1. **Bzowska, A., Kulikowska, E., Darzynkiewicz, E. and Shugar, D.** 1988. Purine nucleoside phosphorylase. *J. Biol. Chem.* **263**, 9212-9217.
2. **Choi, H.S.** 1991. Partial purification and characterization of purine nuclseoside phosphorylase in *Saccharomyces cerevisiae*. *Kor. J. Microbiol.* **29**, 172-178.
3. **Choi, H.S.** 1993. Kinetic analysis of purine nucleoside phosphrylase in *Saccharomyces cerevisiae*. *Kor. J. Microbiol.* **31**, 148-156.
4. **Choi, H.S.** 1996. Purification and characterization of purine nucleoside phosphorylase in *Micrococcus luteus*. *Kor. J. Microbiol.* **34**, 82-89.
5. **Cook, W.J., Ealick, S.E., Bugg, C.E., Stoeckler, J.D. and Parks, R.E., Jr.** 1981. Crystallization and preliminary X-ray investigation of human erythrocytic purine nucleoside phosphorylase. *J. Biol. Chem.*, **256**, 4079-4080.
6. **Dong, M.K., Scott, M.E., Schrier, D.J., Suto, M.J., Sircar, J.C., Black, A., Chang, T. and Gilbertsen, R. B.** 1992. The biochemistry and pharmacology of PD 116124 (8-amino-2'-nordeoxyguanosine), an inhibitor of purine nucleoside phosphorylase (PNP). *J. Pharmacol. Exp. Ther.* **260**, 319-326.
7. **Ealick, S. E., Rule, S.A., Carter, D.C., Greenhough, T.J., Babu, Y.S., Cook, W.J., Habash, J., Helliwell, J. R., Stoeckler, J.D., Parks, R.E., Jr., Chen, S.F. and Bugg, C.E.** 1990. Three-dimensional structure of human erythrocytic purine nucleoside phosphorylase at 3.2 A resolution. *J. Biol. Chem.* **265**, 1812-1820.
8. **Ealick, S.E., Babu, Y.S., Bugg, C.E., Erion, M.D., Guida, W.C., Montgomery, J.A. and Secrist III, J.A.** 1991. Application of crystallographic and modeling methods in the design of purine nucleoside phosphorylase inhibitors. *Proc. Natl. Acad. Sci. USA* **88**, 11540-11544.
9. **Gilbertsen, R.B., Dong, K.K., Kossarek, L.M., Sircar, J.C., Kostlan, C.R. and Conroy, M.C.** 1991. Selective in vitro inhibition of human molt-4 lymphoblasts by the novel purine nucleoside phosphorylase inhibitor, CI-972. *Biochem. Biophys. Res. Commun.* **178**, 1351-1358.
10. **Hoffee, P.A., May, R. and Robertson, B.C.** 1978. Purine nucleoside phosphorylase from Salmonella typhymurium and rat liver. p. 517-524. *In* P.A. Hoffee and M. E. Jones (eds) Methods in Enzymology Vol. 51. Academic Press, London.
11. **Ikezawa, Z., Nishino, T., Murakami, K. and Tsushima, K.** 1978. Purine nucleoside phosphorylase from bovine liver. *Comp. Biochem. Physiol.* **60B**, 111-116.
12. **Jensen, K.F.** 1978. Two purine nucleoside phosphorylases in *Bacillus subtilis*. *Biochim. Biophys. Acta* **525**, 346-356.
13. **Jensen, K.F.** 1976. Purine nucleoside phosphorylase from Escherichia coli and Salmonella typhymurium. *Eur. J. Biochem.* **61**, 377-386.
14. **Kalckar, H.M.** 1947. Differential spectrophotometry of purine compounds by means of specific enzymes. *J. Biol. Chem.* **167**, 429-443.

15. **Krenitsky, T.A., Tuttle, J.V., Miller, W.H., Moorman, A.R., Orr, G.F. and Beauchamp, L.** 1990. Nucleoside analogue inhibitors of purine nucleoside phosphorylase. *J. Biol. Chem.* **265**, 3066-3069.
16. **Lewis, A.S.** 1978. Rabbit brain purine nucleoside phosphorylase. *Arch. Biochem. Biophys.* **190**, 662-670.
17. **Ling, F., Inou, Y. and kimura, A.** 1990. Purification and charaterization of a novel nucleoside phosphorylase from a *Klebsiella* sp. and its use in the enzymatic production of adenine arabinoside. *Appl. Environment. Microbiol.* **56**, 3830-3834.
18. **Ling, F., Inou, Y. and Kimura, A.** 1991. Isolation of adenosine-assimilating bacterium from soil: inducible production of purine nucleoside phosphorylase. *Agric. Biol. Chem.* **55**, 573-575.
19. **Martin, D.W., Jr. and Gelfand, E.W.** 1981. Biochemistry of diseases of immunodevelopment. *Ann. Rev. Biochem.* **50**, 845-877.
20. **Murakami, K. and Tsushima, K.** 1975. Crystallization and some properties of purine nucleoside phosphorylase from chicken liver. *Biochem. Biophys. Acta* **384**, 390-398.
21. **Osborne, W.R.** 1986. Nucleoside kinases in T and B lymphoblasts distinguished by autoradiography. *Proc. Natl. Acad. Sci. USA* **83**, 4030-4034.
22. **Pribe, T., Kandil, O., Nakic, M., Pan, B.F. and Nelson, J.A.** 1988. Selective modulation of antibody response and natural killer cell activity by purine nucleoside analogues. *Cancer Res.* **48**, 4799-4803.
23. **Renout, J.A., Wood, A., Frazer, I.H., Thong, Y.H. and Chalmer, A.H.** 1989. Depressed activities of purine enzymes in lymphocytes of patients infected with human immunodeficiency virus. *Clin. Chem.* **35**, 1478-1481.
24. **Ropp, P.A. and Traut, T.W.** 1991. Allosteric regulation of purine nucleoside phosphorylase. *Arch. Biochem. Biophys.* **288**, 614-620.
25. **Stein, J.M., Stoeckler, J.D., Li, S-Y., Tolman, R.L., MacCoss, M., Chen, A., Karkas, J.D., Ashton, W.T. and Parks, R.E., Jr.** 1987. Inhibition of human purine nucleoside phosphorylase by acyclic nucleosides and nucleotides. *Biochem. Pharmacol.* **36**, 1237-1244.
26. **Stoeckler, J.D.** 1984. Purine nucleoside phosphorylase, p. 35-60. In R.I. Glazer (ed), Developments in Cancer Chemotherapy. CRC Press Inc., Boca Raton, F.L.
27. **Stoeckler, J.D., Ryden, J.B., Parks, R.E., Jr., Chu, M.Y., Lim, M.L., Ren, W.Y. and Klein, R.S.** 1986. Inhibitors of purine nucleoside phosphorylase: effects of 9-deazapurine ribonucleosides and synthesis of 5'-deoxy-5'-iodo-9-deazainosine. *Cancer Res.* **46**, 1774-1778.
28. **Surette, M., Gill, T. and MacLean, S.** 1990. Purification and characterization of purine nucleoside phosphorylase from *Proteus vulgaris*. *Appl. Environment. Microbiol.* **56**, 1435-1439.
29. **Tuttle, J.V. and Krenitsky, T.A.** 1984. Effects of acyalovir and its metabolites on purine nucleoside phosphorylase. *J. Biol. Chem.* **259**, 4065-4069.